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(71) Applicant (for all designated States except US): NYCOMED SALUTAR, INC. [US/US]; 428 Oakmead Parkway, Sunnyvale, CA 94086 (US).

(71) Applicant (for GB only): COCKBAIN, Julian, Roderick, Michaelson [GB/GB]; 27 Ladbroke Road, London W11 3PD (GB).

(72) Inventors; and
(75) Inventors/Applicants (for US only): CARVALHO, Joan [US/US]; 114 Woodleaf Way, Mountain View, CA 94040 (US). FELLMANN, Jere, Douglas [US/US]; 1474 Lexington Way, Livermore, CA 94550 (US). WATSON, Alan, David [AU/US]; 262A Rincon Avenue, Campbell, CA 95008 (US). KOO, Michael [US/US]; 335 Elan Village Lane No. 226, San Jose, CA 95134 (US).

(74) Agents: COCKBAIN, Julian et al.; Frank B Dehn & Co., Imperial House, 15-19 Kingsway, London WC2B 6UZ (GB).

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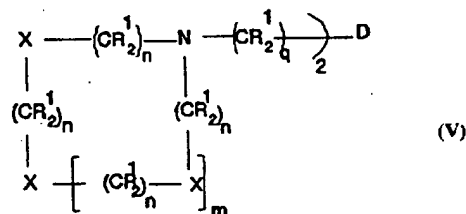
(57) Abstract

This invention relates to dichelants, in particular compounds having two macrocyclic chelant groups linked by a bridge containing an ester or amide bond, especially compounds of formula (V) (wherein each X which may be the same or different is NZ, O or S, at least two Xs being NZ; each Z is a group R^1 or a group CR^1_2Y, at least one Z, and preferably 2 or 3 Zs, on each macrocyclic ring being a group CR^1_2Y; each Y is a group CO_2H, PO_3H, SO_3H, $CONR^1_2$, $CON(OR^1)R^1$, CNS or $CONR^1NR^1_2$, preferably COOH; m is 0 or 1 or 2, preferably 1; each n is 2 or 3, preferably 2; q is 1 or 2, preferably 1; each R^1 which may be the same or different is a hydrogen atom or an alkyl group optionally substituted by one or more hydroxy and/or alkoxy groups; and D is a bridging group, other than an unsubstituted carbonylaminoethylaminocarbonyl group, having a molecular weight of less than 1000, preferably less than 500, joining two macrocyclic rings via at least one amide or ester bond) and salts and metal chelates thereof.

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POLYAZACYCLOALKANES AS DICHELANTS

FIELD OF THE INVENTION

The present invention relates to dichelants, that is
chelating agents capable of complexing two metal ions
simultaneously, and to chelates and salts thereof and
their use in diagnostic and therapeutic compositions,
especially as contrast enhancing agents in diagnostic
medical imaging.

BACKGROUND OF THE INVENTION

The medical use of chelants is now well established, for
example as stabilisers for pharmaceutical preparations,
as antidotes for poisonous heavy metal species, as
carriers for diagnostically or therapeutically useful
metal ions, for example in contrast media for use in
magnetic resonance, X-ray or ultrasound imaging or in
scintigraphy.

For such diagnostic agents, it is generally important
that the chelate complexes should be stable both
kinetically and thermodynamically and for this reason
there has been much interest in the macrocyclic
polyamine-based chelates, in particular DOTA and its
derivatives and analogues, which form very stable
complexes with the lanthanide metal ions such as
gadolinium and dysprosium which are favoured diagnostic
metal ions for magnetic resonance imaging due to their
relatively large effects on the relaxation times (e.g. T_1
and T_2^*) of neighbouring water protons.

The paramagnetic lanthanide metal ions useful as MR
imaging contrast agents are relatively toxic and for

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clinical use must be administered in a form which allows
little or no release of the metal for subsequent
biological uptake and retention. For this reason, from
the early years of MR contrast agents, the use of stable
chelate complexes has been proposed. Thus the first
commercial lanthanide based MR imaging contrast agent,
Magnevist, contained GdDTPA, a complex with a high
stability constant which following parenteral
administration is excreted relatively rapidly by
glomerular filtration with the gadolinium still in the
chelate complex.

GdDOTA has an even higher pK_{ML} and thus was also a prime
candidate for consideration as an MR imaging contrast
agent. DOTA (1,4,7,10-tetraazacyclododecane-
N,N',N",N"'-tetraacetic acid) and HPDO3A (1-(2-
hydroxypropyl)-4,7,10-tetraazacyclododecane-N,N',N"-
triacetic acid) have indeed been proposed as chelants
for MR imaging contrast agents and GdDOTA and GdHPDO3A
have been commercially developed by companies active in
this field.

The lanthanide metals generally have a stable +3
oxidation state and DOTA with its four carboxylic acid
groups results in a charged complex, i.e. GdDOTA⁻,
requiring a counterion. Analogous uncharged complexes
may be produced by eliminating one of DOTA's nitrogen-
attached carboxymethyl groups or by replacing it by a
non-ionizing group, i.e. by using a chelant such as DO3A
(1,4,7,10-tetraazacyclododecane-N,N',N"-triacetic acid)
or HPDO3A.

Contrast media based on such non-ionic, or overall
charge neutral, complexes have lower osmolalities for a
given metal ion concentration and can demonstrate other
improved properties relative to the analogous charged
complexes. Moreover, the ring nitrogen "freed" by

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removal of the carboxymethyl group in moving from DOTA
to DO3A can of course be substituted by groups which can
act to enhance the hydrophilicity or lipophilicity or
other biodistribution affecting properties of the
chelate.

Recently, there has been growing interest in the use of
chelants capable of chelating more than one metal ion
per chelant molecule as carriers for paramagnetic or
heavy metal ions for MR or X-ray imaging contrast
agents. These polychelants offer several advantages
over the monochelants such as DTPA, DO3A or DOTA. Thus
for example, the osmolality at a given metal
concentration can be reduced still further, the
simultaneous delivery of a plurality of metal ions to a
target site can be facilitated, and more efficient
contrast agents can be produced.

Polychelants range from dichelants through oligochelants
to true polychelants having perhaps hundreds of chelant
moieties per molecule. Many such compounds have been
described but there is still a need for polychelants,
and in particular oligochelants and especially
dichelants, having improved properties in terms for
example of relaxivity, stability, biodistribution,
biotolerability, viscosity, solubility and osmolality.

Particular macrocyclic dichelants described in the
literature include the DO3A dimers of formula I, II, III
and IV whose preparation has been described by Nycomed
Salutar in WO-A-91/05762 and Schering AG in EP-A-255471
and EP-A-485045 (US-A-5277895) and elsewhere.

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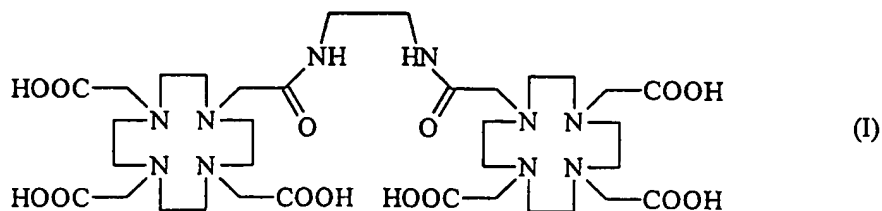
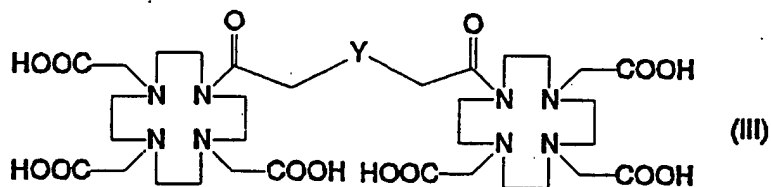(described by Nycomed Salutar in WO-A-91/05762 and USSN 07/855028)
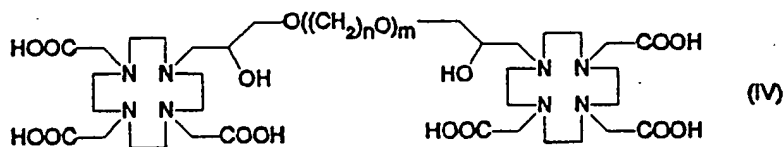

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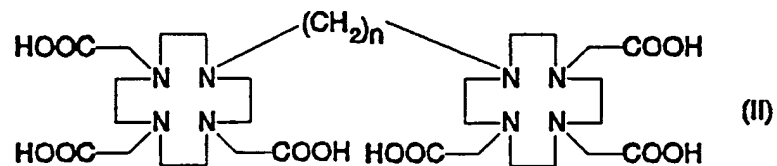
(where n = 2, 3, 5 or 6)

(described by Schering AG in EP-A-255471 and in a poster presented at the European Congress of NMR in Medicine and Biology at Strasbourg in May 1990)

(III)

(where Y is [-N(CH$_2$COOH)CH$_2$CH$_2$N(CH$_2$COOH)CH$_2$CH$_2$N(CH$_2$COOH)]$_a$ and a = 0 or 1)

(described by Schering AG in EP-A-255471)

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(where n =2 or 4 and m =0 or 1)

(described by Schering AG in EP-A-485045 (US-A-5277895).

All of these macrocyclic chelant dimers have the general
formula DO3A'-L-DO3A' where DO3A' is a ring nitrogen
deprotonated DO3A residue and L is a linker group.

With lanthanides such as gadolinium, these macrocyclic
dimers will produce non-ionic dichelates and these
compounds have been found to possess high relaxivity.
Thus for example the T1 relaxivities of the
bisgadolinium chelates of the compounds of formula II
are almost double the T1 relaxivity of GdDO3A.

SUMMARY OF THE INVENTION

We have now found that dichelant compounds having
improved properties are produced if the linker groups
incorporate ester or amide functionalities, and
especially where the linkers comprise carbonyl-attached
alkylene groups in which two or more of the methylene
groups are replaced by nitrogen or oxygen atoms.

Thus viewed from one aspect the invention provides a
polychelant of formula V

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$$X \text{——}(CR^1_2)_n\text{——}N\text{——}\left(\left(CR^1_2\right)_q\right)\text{——}D$$

(structure with vertical branches:)

$(CR^1_2)_n$ ... $(CR^1_2)_n$

$X \text{——}\left[\text{(}CR^1_2\text{)}_n\text{——}X\right]_m$

(wherein each X which may be the same or different is
NZ, O or S, at least two Xs being NZ;
each Z is a group R^1 or a group CR^1_2Y, at least one Z,
and preferably 2 or 3 Zs, on each macrocyclic ring being
a group CR^1_2Y;
each Y is a group CO_2H, PO_3H, SO_3H, $CONR^1_2$, $CON(OR^1)R^1$,
CNS or $CONR^1NR^1_2$, preferably COOH;
m is 0 or 1 or 2, preferably 1; each n is 2 or 3,
preferably 2; q is 1 or 2, preferably 1;
each R^1 which may be the same or different is a hydrogen
atom or an alkyl group optionally substituted by one or
more hydroxy and/or alkoxy groups;
and D is a bridging group, other than an unsubstituted
carbonylaminoethylaminocarbonyl group, having a
molecular weight of less than 1000, preferably less than
500, joining two macrocyclic rings via at least one
amide or ester bond) and salts and metal chelates
thereof.

Viewed from a further aspect, the present invention
provides a diagnostic or therapeutic agent comprising a
metal chelate, whereof the chelating entity is the

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residue of a compound according to the present
invention, together with at least one pharmaceutical or
veterinary carrier or excipient, or adapted for
formulation therewith or for inclusion in a
pharmaceutical formulation for human or veterinary use.

Viewed from another aspect, the present invention
provides a detoxification agent comprising a chelating
agent according to the invention in the form of a weak
complex or salt with a physiologically acceptable
counterion, together with at least one pharmaceutical or
veterinary carrier or excipient, or adapted for
formulation therewith or for inclusion in a
pharmaceutical formulation for human or veterinary use.

Viewed from a further aspect, the present invention
provides a method of generating enhanced images of the
human or non-human animal body, which method comprises
administering to said body an effective amount of a
diagnostic agent comprising a metal chelate of compound
of formula V, or a salt thereof, and generating an image
of at least part of said body to which said chelate
distributes, wherein said metal is paramagnetic,
radioactive or X-ray opaque.

Viewed from a further aspect, the present invention
provides a method of radiotherapy practised on the human
or non-human animal body, which method comprises
administering to said body an effective amount of a
chelate of a radioactive metal species with a chelating
agent of formula V, or a salt thereof.

Viewed from a further aspect, the present invention
provides a method of heavy metal detoxification
practised on the human or non-human animal body, which
method comprises administering to said body an effective
amount of a chelating agent of formula V or a

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physiologically tolerable salt or weak complex thereof.

Viewed from a still further aspect, the present
invention provides a process for the preparation of the
metal chelates of the invention which process comprises
admixing in a solvent a compound of formula V or a salt
(e.g. the sodium salt) or chelate thereof together with
an at least sparingly soluble compound of said metal,
for example a chloride, oxide, acetate or carbonate.

DETAILED DESCRIPTION

In the compounds of the invention, the macrocyclic
$N(CR^1_2)_n(X(CR^1_2)_n)_{m+2}$ rings preferably have 9 to 14 ring
atoms, the ring heteroatoms especially preferably being
either all nitrogen or being one oxygen and three
nitrogens. The alkylene ring segments $(CR^1_2)_n$ preferably
are all $(CR^1_2)_2$ groups or in the case of an N_4 macrocycle
the alkylene segments may alternatively be alternating
$(CR^1_2)_3$ and $(CR^1_2)_2$ groups. Thus the preferred
macrocyclic skeletons are those of formulae VI

(VI)
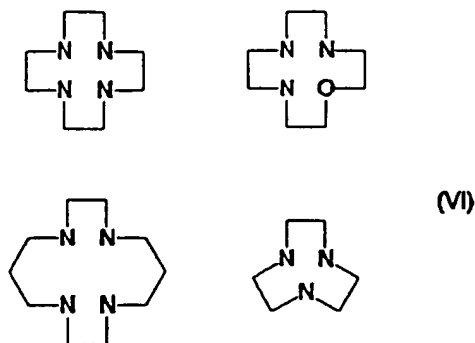
The bridging group D is conveniently a group of formula
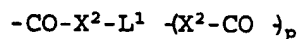
$$-CO-X^2-L^1 \; (X^2-CO \;)_p$$

where p is 0 or 1,

X^2 is O or NR^2,

R^2 is a hydrogen atom or a hydroxy, OR^1 or NR^1_2 group or
an alkyl group optionally interrupted by oxygen, sulphur
or nitrogen atoms or by carbonyl or aryl groups and
optionally substituted by hydroxyl, amine or aryl
groups, or R^2 contains a functional group for attachment
to a biomolecule or macromolecule, or two R^2 groups
together form a bridging linker group, e.g. a group L^1,
and L^1 which provides a chain of at least two atoms
linking two X^2 groups or at least one atom linking an X^2
group and a $(CR^1_2)_q$ moiety (wherein R^1 and q are as
hereinbefore defined), is a straight chain, branched or
cyclic alkylene group or a combination of such groups,
optionally substituted and optionally being interrupted
by oxygen, sulphur or nitrogen atoms or by aryl or
carbonyl groups.

The linker group L^1 will preferably be a linear, branched
or cyclic alkylene group or a combination thereof or a
combination of arylene and alkylene groups, for example
providing a linking backbone 1 to 50 atoms long but

preferably 2 to 25, and especially 2 to 10 atoms long in
total on any one unbranched segment. The carbon
backbone in such linker groups may be interrupted by
heteroatoms such as nitrogen, oxygen and sulphur, and
may carry bridging groups, thereby creating homo- or
heterocyclic rings within the linker group. Where this
occurs, the rings created will preferably be 3 to 12,
especially 5 to 8 and, particularly, 6 membered rings.
Moreover the rings and the linear segments of the linker
group may optionally be unsaturated and may optionally
carry one or more substituents selected from alkyl,
hydroxy, alkoxy, amine, aryl and substituted aryl groups
as well as non-hydrogen R^1 groups or additional chelating
groups, eg. Y groups especially carbonyl and SO_3H groups,
and groups such as for example long chain (eg. C_{10-20})
alkyl, aryl or polyaryl groups which are suitable for
liposomal incorporation of the compound of formula V or
groups, such as isothiocyanate groups, for attachment of
the compound of formula V to a biomolecule, polymer,
dendrimer or other macromolecule, for example to create
a bifunctional chelant.

The bridging group D in the compounds of the invention
may, as indicated above, serve to link together two
chelant moieties, thereby holding together the dichelate
structure. Besides filling this role as linker or
spacer of chelant sites, the bridging group can be so
selected as to yield a product having other desired
characteristics. For example it is possible to increase
hydrophilicity, lipophilicity, or tissue specificity of
the end product by attaching to or incorporating within
the bridging group, groups which are hydrophilic,
lipophilic, or tissue targeting. In this way, the
overall charge of the chelate structure, or the overall
lipophilicity, or tissue targeting can be controlled.

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In the compounds of formula V, alkyl moieties preferably
have 1 to 6, especially 1 to 4 carbon atoms unless
otherwise specified, and aryl moieties are preferably
phenyl groups.

Particularly preferred compunds of formula V include
those of formula VII

$$M-CH_2CO)_2-D' \qquad\qquad (VII)$$

where M is a nitrogen attached triaza, tetraaza,
triazaoxa or triazathia-cycloalkane of formula VI having
at least one and preferably two ring nitrogens
substituted by CH_2COOH groups and having any remaining
ring nitrogen substituted by a group R^3, M preferably
being a group of formula VI having two or more,
preferably three, ring heteroatoms substitued by CH_2COOH
groups; R^3 (which for tetraheteroatom rings is preferably
at the ring heteroatom remote from the ring attachment
nitrogen) is a hydrogen atom, or an alkyl group
optionally mono or polysubstituted or hydroxyl or alkoxy
groups (eg. hydroxyalkyl, polyhydroxyalkyl, alkoxyalkyl,
polyalkoxyalkyl, hydroxyalkoxyalkyl,
hydroxypolyalkoxyalkyl, polyethylene glycol etc.) and
optionally interrupted by arylene or substituted arylene
groups; and CO-D'-CO is a bridging group D as discussed
above.

Preferred bridging groups D in the dichelant compounds
of the invention include those of the formulae:

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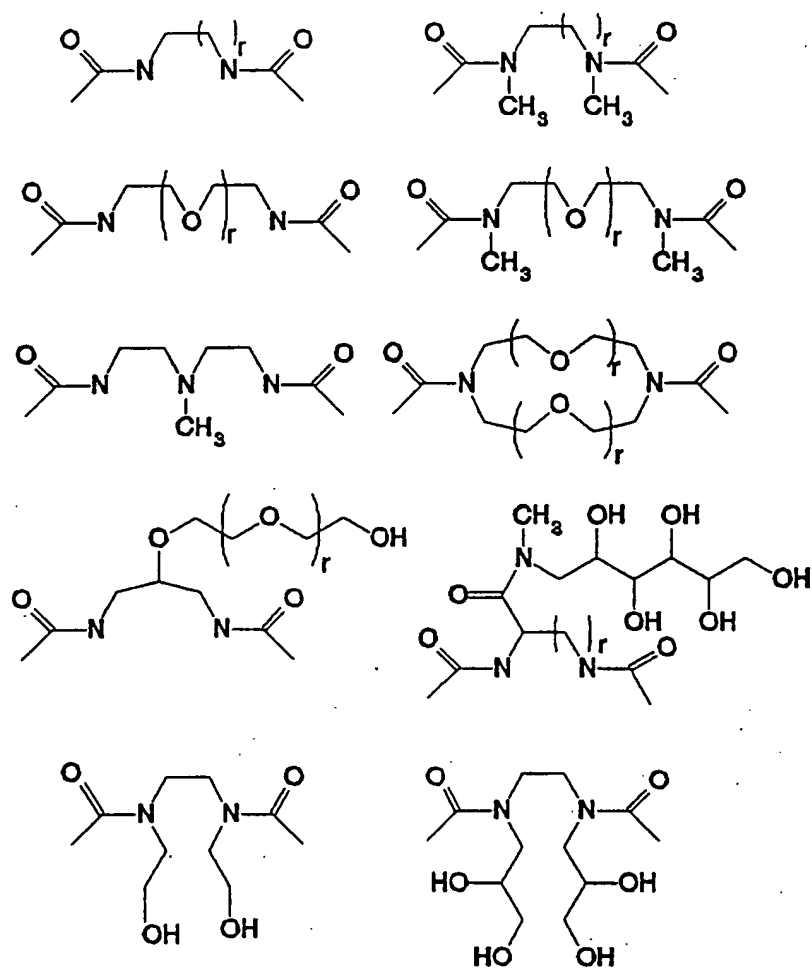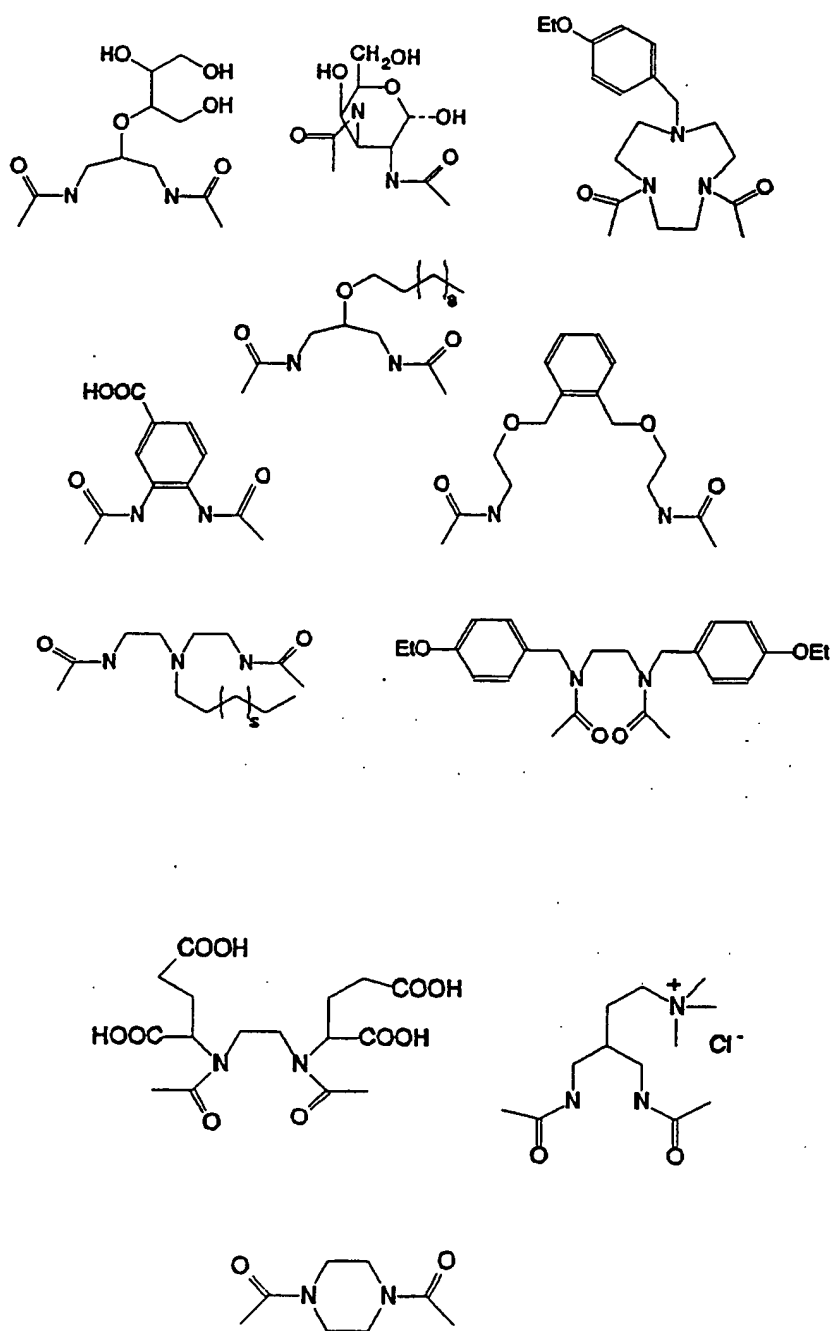
where r is an integer having a value of 1 to 6,
especially 1, 2 or 3, and s is an integer having a value
of 1 to 20, especially 1 to 15.

Especially preferred are bridging groups containing
ether oxygens, for example
$COOCH_2CH_2OCH_2CH_2NHCH_2CH_2OCH_2CH_2OCO$, as these may be
associated with particularly low toxicity. Also
especially preferred are bridging groups containing
amide linkages, for example $CONHCH_2CH_2OCH_2CH_2OCH_2CH_2NHCO$.

The D groups can, as seen above, carry substituent side
chains and where D is a carbonylaminoethylaminocarbonyl
group it will be so substituted, e.g. with optionally
hydroxylated, optionally carboxylated alkyl or oxaalkyl
groups optionally interrupted by carbonyamino or arylene
functions.

Preferred compounds according to the invention also
include the polyhydroxylated dichelants, e.g. where D or
R^1 groups are hydroxylated, as they can present a better
toxicity profile than their non-hydroxylated
counterparts. Lipophilic analogs are also of interest
due to the potential for increased liver uptake;
moreover the long chain lipophilic analogs have the
ability to be incorporated into liposomes for use as
blood pool agents and for targetted delivery to specific
organs or tissues. Indeed chelate compounds according
to the invention, in particular the lipophilic analogs,
are of particular interest as blood pool agents where
they exhibit prolonged plasma half lifes.

The compounds of the invention may be prepared by
conventional synthetic techniques, conveniently starting
from the corresponding N-unsubstituted or N-
carboxymethylated polyazacycloalkanes, condensing these
to a bifunctional linker molecule, generally after

protection of one or more of the ring nitrogens or N-
carboxymethyl groups, followed by deprotection and if
required introduction of functional groups at the ring
nitrogens.

Where the dichelant is asymmetrical, it can of course be
constructed by conjugating one macrocycle to a
monoprotected bifunctional linker molecule,
deprotecting, and conjugating a second macrocycle to the
macrocycle-linker intermediate. For such purposes, the
bifunctional linker molecule may of course itself be
asymmetric to allow one end to conjugate directly to a
macrocycle ring nitrogen and the other to conjugate to a
macrocycle side chain, e.g. a carboxymethyl group or a
reactive derivative thereof.

Thus viewed from a further aspect the invention also
provides a process for the preparation of the compounds
of the invention, said process comprising at least one
of the following steps:

(a) reacting a compound of formula V wherein at least
one group X is a group NH, with a compound of formula
VIII

$$Lv-R^4 \qquad (VIII)$$

(where Lv is a displaceable leaving group, for example a
halogen atom or a substituted sulphonyloxy group, such
as chlorine, bromine, iodine, methanesulphonyloxy,
phenysulphonyloxy or p-toluenesulphonyloxy groups) and R^4
is a group CR^1_2Y or group R^1 other than hydrogen);

(b) reacting compounds of formulae X and/or XI

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$$X \underbrace{\qquad}(CR^1_2)_n \underbrace{\qquad} N \underbrace{\qquad}(CR^1_2)_q \underbrace{\qquad} COOH$$

$$(CR^1_2)_n \qquad\qquad (CR^1_2)_n$$

$$X \underbrace{\left[(CR^1_2)_n \underbrace{\qquad} X \right]_m} \qquad (X)$$

$$X \underbrace{\qquad}(CR^1_2)_n \underbrace{\qquad} NH$$

$$(CR^1_2)_n \qquad\qquad (CR^1_2)_n$$

$$X \underbrace{\left[(CR^1_2)_n \underbrace{\qquad} X \right]_m} \qquad (XI)$$
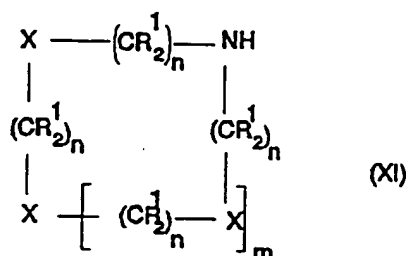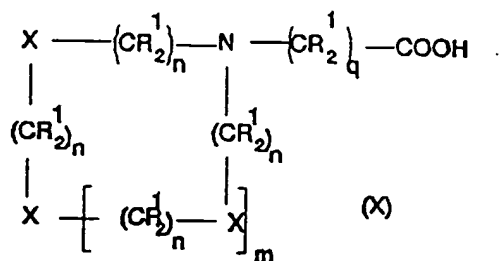
(wherein X, R^1, n, q and m are as hereinbefore defined)
or an activated derivative, e.g. halide, thereof with a
linker molecule of formula IX

$$\text{Lv}(\text{CO-X}^2\ )L^1-(X^2\text{-CO})_t\text{Lv} \qquad\qquad (IX)$$

or formula IXa

$$\text{Lv-}(CR^1_2)_q\text{-}(\text{CO-X}^2\ )L^1\text{-}(X^2\text{-CO})_t\text{-}(CR^1_2)_q\text{-Lv} \qquad\qquad (IXa)$$
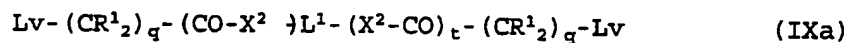
(wherein X^2, L^1, R^1 and q are as hereinbefore defined, t
is 0 or 1, and each Lv is a displaceable leaving group,
one optionally being protected prior to conjugation of
the second compound of formula X or XI);

(c) converting a compound of formula X into a
corresponding acid chloride and reacting with a suitable
diamine;

(d) metallating or transmetallating a compound of

formula V or a chelate thereof;

(e) converting a compound of formula V or a chelate thereof into a base or acid addition salt thereof or converting a salt into the free acid or base; and

(f) performing at least one of steps (a) to (d) above using reagents with protected functional groups and subsequently removing the protecting groups.

The starting compounds of formulae VIII, IX, X and XI are either known from the literature or can be produced by conventional synthetic techniques. The starting compounds of formula VIII used in step (a) can be prepared by the process of step (b).

As indicated above, during the reaction, functional groups present in the starting materials but not involved in the particular process steps may be protected, for example to avoid unwanted substitution or polymerisation. Conventional protection and deprotection techniques may be used (see for example "Protective Groups in Organic Synthesis" by T. W. Greene, Wiley-Interscience, NY, 1981 and "Protective Groups in Organic Chemistry" by JFW McOmie, Plenum, London, 1973). Suitable protecting groups for carboxyl groups include ester functions, for ring nitrogens alkyl, borane or organometallic functions, for hydroxyl groups acyl functions. The protecting groups will be removed by standard techniques, for example hydrolysis, hydrogenolysis, etc. after the reactions step is complete.

Salt and chelate formation may be effected by conventional techniques, e.g. as described in the above mentioned patent publications.

The skeletons of the macrocyclic chelant groups or, more
preferably, of the linker moiety, may be derivatised to
enhance properties of the overall chelant, for example
to include hydrophilic or lipophilic groups or
biologically targetting groups or structures. Examples
of macromolecules, biomolecules and macrostructures to
which the polymeric chelant may be conjugated in this
way include polymers (such as polylysine or
polyethyleneglycol), dendrimers (such as first to sixth
generation starburst dendrimers and in particular PAMAM
dendrimers), polysaccharides, proteins, antibodies or
fragments thereof (especially monoclonal antibodies or
fragments such as Fab fragments), glycoproteins,
proteoglycans, liposomes, aerogels, peptides, hormones,
steroids, microorganisms, human or non-human cells or
cell fragments, cell adhesion molecules (in particular
nerve adhesion molecules such as are described in WO-A-
92/04916), other biomolecules, etc). Generally such
derivatisation will be achieved most conveniently by the
introduction of alkyl- or aralkyl-carried functions, to
which the macromolecule, biomolecule, etc. can be bound
either directly or via a linker molecule, for example a
bi- or polyfunctional acid, activated acid or oxirane.

In the case of conjugation to dendrimers, the dendrimer
carriers can be produced by standard techniques, for
example as desribed by Tomalia and by Nycomed Salutar in
Angew Chem Int Ed Eng 29:138 (1990), WO-A-88/01178, WO-
A-90/12050 and WO-A-93/06868 and the references cited
therein.

Such macromolecular derivatives of the compounds of
formula V and the metal chelates and salts thereof form
a further aspect of the present invention.

The linkage of a compound of formula V to a
macromolecule or backbone polymer may be effected by the

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methods of Nycomed Salutar (WO-A-90/12050) or by any of
the conventional methods such as the carbodiimide
method, the mixed anhydride procedure of Krejcarek et
al. (see Biochemical and Biophysical Research
Communications 77: 581 (1977)), the cyclic anhydride
method of Hnatowich et al. (see Science 220: 613 (1983)
and elsewhere), the backbone conjugation techniques of
Meares et al. (see Anal. Biochem. 142: 68 (1984) and
elsewhere) and Schering (see EP-A-331616 for example)
and by the use of linker molecules as described for
example by Nycomed Imaging in WO-A-89/06979 (US-A-
5208324).

Salt and chelate formation may be performed in a
conventional manner. The chelating agents of formula V
may be used in detoxification or in the formation of
metal chelates, chelates which may be used for example
in or as contrast agents for _in vivo_ or _in vitro_
magnetic resonance (MR), X-ray or ultrasound diagnostics
(e.g. MR imaging and MR spectroscopy), or scintigraphy
or in or as therapeutic agents for radiotherapy, and
such uses of these metal chelates form a further aspect
of the present invention.

Salts or chelate complexes of the compounds of the
invention containing a heavy metal atom or ion are
particularly useful in diagnostic imaging or therapy.
Especially preferred are salts or complexes with metals
of atomic numbers 20-32, 42-44, 49 and 57 to 83,
especially Gd, Dy and Yb. For use as an MR-diagnostics
contrast agent, the chelated metal species is
particularly suitably a paramagnetic species, the metal
conveniently being a transition metal or a lanthanide,
preferably having an atomic number of 21-29, 42, 44 or
57-71. Metal chelates in which the metal species is Eu,
Gd, Dy, Ho, Cr, Mn or Fe are especially preferred and
Gd^{3+}, Mn^{2+} and Dy^{3+} are particularly preferred.

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Chelates of ions of these metals specifically listed
above with chelants of formula V or their salts with
physiologically tolerable counterions are particularly
useful for the diagnostic imaging procedures mentioned
herein and they and their use are deemed to fall within
the scope of the invention and references to chelates of
compounds of formula V herein are consequently to be
taken to include such chelates.

The bislanthanide complexes of the compounds of formula
V are especially preferred.

For diagnostic imaging purposes it is particularly
important that the metal chelate complex be as stable as
possible to prevent dissociation of the complex in the
body.

In magnetic resonance imaging (MRI) it is frequently
desirable to be able to target certain organs or
tissues. In particular there is a need for improved
hepatobiliary imaging MR contrast agents. Chelates of
paramagnetic metals with compounds of formula V carrying
one or more lipophilic groups are particularly suited
for use as hepatobiliary MR contrast agents, since the
presence of the lipophilic group will promote uptake by
hepatocytes. By linking the lipophilic group to the
molecule via a readily hydrolysable linking group such
as an ester, the reabsorption after excretion to the
intestine can be prevented.

The paramagnetic metal chelates of compounds of formula
I, especially the chelates of high spin metal ions such
as Gd^{3+} and more especially Dy^{3+}, are particularly
suitable for use as magnetic susceptibility (MS)
contrast agents (T_2 or T_2^{*} contrast agents) in MR imaging
and other MR investigations. The use of paramagnetic
metal mono-chelates is discussed by Villringer et al. in

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Mag. Reson. Med. 6:164-174 (1988) and by Kucharczyk et
al. in US-A-5190744 and WO-A-91/14186. Using the
dimeric chelates of the present invention, smaller
volumes of contrast medium can be administered to
achieve the same MS effect thus allowing for more
effective bolus dosing. Moreover, while for
monochelates high susceptibility paramagnetic centres
such as Dy(III) generally have to be used in MS studies,
by using the dimeric chelates of the invention a wider
range of paramagnetic metal centres, including in
particular Gd(III) become useful.

For certain hepatobiliary imaging purposes it is
desirable that the lipophilic contrast agent be
precipitated as particles which can be taken up by
Kupffer cells in the liver. In such cases it is
preferred to use chelates of Dy^{3+} with lipophilic
compounds of formula V in conjunction with an imaging
system utilising the magnetic susceptibility properties
of the contrast agent; Kupffer cells in the liver are
scarce and the contrast achievable using chelates with
the gadolinium normally used in conventional MR imaging
(i.e. as a T_1 relaxation agent) is generally
insufficient. Such magnetic susceptibility agents form
an important embodiment of the invention.

For use as contrast agents in MRI, the paramagnetic
metal species is conveniently non-radioactive as
radioactivity is a characteristic which is neither
required nor desirable for MR-diagnostic contrast
agents. For use as X-ray or ultrasound contrast agents,
the chelated metal species is preferably a heavy metal
species, for example a non-radioactive metal with an
atomic number greater than 37, preferably greater than
50, e.g. Dy^{3+}.

For use in scintigraphy and radiotherapy, the chelated

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metal species must of course be radioactive and any
conventional complexable radioactive metal isotope, such
as ^{99m}Tc, ^{67}Ga or ^{111}In for example, may be used. For
radiotherapy, the chelating agent may be in the form of
a metal chelate with for example ^{153}Sm, ^{67}Cu or ^{90}Y.

For use in detoxification of heavy metals, the chelating
agent should be in salt form with a physiologically
acceptable counterion, e.g. sodium, calcium, ammonium,
zinc or meglumine, e.g. as the sodium salt of the
chelate of the compound of formula V with zinc or
calcium.

Where the metal chelate carries an overall charge, such
as is the case with the prior art Gd DTPA, it will
conveniently be used in the form of a salt with a
physiologically acceptable counterion, for example an
ammonium, substituted ammonium, alkali metal or alkaline
earth metal (e.g. calcium) cation or an anion deriving
from an inorganic or organic acid. In this regard,
meglumine salts are particularly preferred.

The diagnostic and therapeutic agents of the present
invention may be formulated with conventional
pharmaceutical or veterinary formulation aids, for
example stabilizers, antioxidants, osmolality adjusting
agents, buffers, pH adjusting agents, etc. and may be in
a form suitable for parenteral or enteral
administration, for example injection or infusion or
administration directly into a body cavity having an
external escape duct, for example the gastrointestinal
tract, the bladder or the uterus. Thus the agent of the
present invention may be in a conventional
pharmaceutical administration form such as a tablet,
capsule, powder, solution, suspension, dispersion,
syrup, suppository, etc; however, solutions, suspensions
and dispersions in physiologically acceptable carrier

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media, for example water for injections, will generally
be preferred.

The compounds according to the invention may therefore
be formulated for administration using physiologically
acceptable carriers or excipients in a manner fully
within the skill of the art. For example, the compounds,
optionally with the addition of pharmaceutically
acceptable excipients, may be suspended or dissolved in
an aqueous medium, with the resulting solution or
suspension then being sterilized. Suitable additives
include, for example, physiologically biocompatible
buffers (as for example, tromethamine hydrochloride),
additions (e.g. 0.01 to 10 mole percent) of chelants
(such as, for example, DTPA, DTPA-bisamide or
non-complexed chelants of formula I) or calcium chelate
complexes (as for example calcium DTPA,
CaNaDTPA-bisamide, calcium salts or chelates of chelants
of formula VII), or, optionally, additions (e.g. 1 to 50
mole percent) of calcium or sodium salts (for example,
calcium chloride, calcium ascorbate, calcium gluconate
or calcium lactate combined with metal chelate complexes
of chelants of formula I and the like).

If the compounds are to be formulated in suspension
form, e.g., in water or physiological saline for oral
administration, a small amount of soluble chelate may be
mixed with one or more of the inactive ingredients
traditionally present in oral solutions and/or
surfactants and/or aromatics for flavouring.

For MRI and for X-ray imaging of some portions of the
body the most preferred mode for administering metal
chelates as contrast agents is parenteral, e.g.
intravenous administration. Parenterally administrable
forms, e.g. intravenous solutions, should be sterile and
free from physiologically unacceptable agents, and

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should have low osmolality to minimize irritation or
other adverse effects upon administration, and thus the
contrast medium should preferably be isotonic or
slightly hypertonic. Suitable vehicles include aqueous
vehicles customarily used for administering parenteral
solutions such as Sodium Chloride Injection, Ringer's
Injection, Dextrose Injection, Dextrose and Sodium
Chloride Injection, Lactated Ringer's Injection and
other solutions such as are described in Remington's
Pharmaceutical Sciences, 15th ed., Easton: Mack
Publishing Co., pp. 1405-1412 and 1461-1487 (1975) and
The National Formulary XIV, 14th ed. Washington:
American Pharmaceutical Association (1975). The
solutions can contain preservatives, antimicrobial
agents, buffers and antioxidants conventionally used for
parenteral solutions, excipients and other additives
which are compatible with the chelates and which will
not interfere with the manufacture, storage or use of
products.

Where the diagnostic or therapeutic agent comprises a
chelate or salt of a toxic metal species, e.g. a heavy
metal ion, it may be desirable to include within the
formulation a slight excess of the chelating agent, e.g.
as discussed by Schering in DE-A-3640708 (US-A-5098692),
or more preferably a slight excess of the calcium salt
of such a chelating agent.

For MR-diagnostic examination, the diagnostic agent of
the present invention, if in solution, suspension or
dispersion form, will generally contain the metal
chelate at concentration in the range 1 micromole to 1.5
mole per litre, preferably 0.1 to 700mM. The diagnostic
agent may however be supplied in a more concentrated
form for dilution prior to administration. The
diagnostic agent of the invention may conveniently be
administered in amounts of from 10^{-3} to 3 mmol of the

metal species per kilogram of body weight, e.g. about 1
mmol lanthanide (e.g. Dy or Gd)/kg bodyweight.

For X-ray examination, the dose of the contrast agent
should generally be higher and for scintigraphic
examination the dose should generally be lower than for
MR examination. For radiotherapy and detoxification,
conventional dosages may be used.

The disclosures of all of the documents mentioned herein
are incorporated by reference.

The present invention will now be illustrated further by
the following non-limiting Examples. All ratios and
percentages given herein are by weight and all
temperatures are in degrees Celsius unless otherwise
indicated.

Example 1

Synthesis of N,N'-Bis[1,4,7-tris-(carboxymethyl)-1,4,7,10-tetraazacyclo-dodecan-1-methylcarbonyl]-piperazine and the gadolinium complex thereof

(a)   1,4,7,10-Tetraazacyclododecane (cyclen)

To a suspension of tetraaza-12-crown-4
tetrahydrochloride (66.6 g, 0.209 mol, Parish, Inc.) in
chloroform (2 L), was bubbled NH_3 (g) through a gas
dispersion tube for 1 hour. The solution was allowed to
stir overnight and the white solid was filtered off and
washed with $CHCl_3$ (4 x 100 mL). The combined filtrate
was concentrated in vacuo to a white solid which was
washed with diethyl ether (4 x 50 mL) and dried under
vacuum at ambient temperature. A second crop of the
free base was isolated from the ether washes to give a
combined yield of 35.1 g (97.5%). ^1H NMR ($CDCl_3$): δ
2.32 (s, 4 H), 2.70 (s, 16 H).

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(b)   1,4,7-Tris-(tert-butoxy-carbonylmethyl)- 1,4,7,10-tetraazacyclododecane-hydrobromide

Cyclen (35.0 g, 0.203 mole) (Example 1(a)) was dissolved in N,N-dimethylacetamide (DMA, 600 mL) under nitrogen. Sodium acetate (50.0 g, 0.61 mol) was added at once and the mixture was allowed to stir for 0.5 hour. A solution of t-butylbromoacetate (118.9 g, 0.61 mol) in DMA (150 mL) was added dropwise from an addition funnel over a 7 hour period. The reaction mixture was allowed to stir for 19 days at ambient temperature under nitrogen during which time a white solid precipitated from solution. The white solid was then filtered off, washed with chilled DMA (75 mL) and ethyl acetate (100 mL), and dried under vacuum at 50°C. The filtrate was concentrated to approximately 500 mL and a second crop of white solid was collected in a similar manner. The combined solids (80.2 g + 38.4 g) were taken up in CHCl$_3$ (600 mL) and washed with deionized H$_2$O (4 x 100 mL). The organic layer was dried (Na$_2$SO$_4$), filtered, and concentrated to a light yellow oil. A white solid was obtained by addition of ethyl acetate to the oil. The solid was collected by filtration, washed with diethyl ether (2 x 75 mL) , and dried under vacuum at 45°C to give 67.4 g (55.7%) of the title monohydrobromide salt. Additional product can be recovered from the filtrates if desired. ^{1}H NMR (CDCl$_3$): δ 1.42 (s, 27 H), 1.71 (s, 2 H), 2.86 (m, 12 H), 3.06 (br s, 4 H), 3.25 (s, 2 H), 3.34 (s, 4 H). Anal. Calcd (found) for C$_{26}$H$_{51}$N$_4$O$_6$Br: C, 52.43 (52.47); H, 8.63 (8.48); N, 9.40 (9.50); Br, 13.42 (12.92).

(c)   Piperazine bis(bromoacetamide)

To a 1 L three-necked flask equipped with a magnetic
stir bar, reflux condenser and addition funnel, was
added bromo acetyl bromide (82.8 g, 0.41 mole) in CHCl₃
(170 mL). The addition funnel was charged with a
solution of piperazine (0.2 mole, 17.2 g) and
triethylamine (70 mL) in CHCl₃ (180 mL). The flask was
chilled to -15°C by means of a CH₃CN/liquid N₂ bath, and
the amine was slowly added to the acid bromide. After
the addition was complete the mixture was allowed to
warm to ambient temperature and was stirred for 1 hour.
The flask was then cooled to 0°C and H₂O (100 mL) was
slowly added. The mixture was diluted with CHCl₃ (500
mL) and the layers were separated. The organic layer
was extracted with H₂O (5 x 50 mL), 0.05 N NaOH   (5 x 50
mL) and H₂O (200 mL), and dried (Na₂SO₄). The dark
orange solution was filtered and concentrated to a beige
solid. The material was purified by filtration through
a bed of silica gel. The product eluted with 2 - 5%
methanol/CH₂Cl₂. After combining and concentrating the
desired fractions, 15.0 g (49.2%) of a white solid was
obtained. The title product was recrystallized from
warm 2-propanol (400 mL) affording 9.95 g. ¹H NMR
(CDCl₃): δ 3.61 (dt, 6 H), 3.85 (s, 4 H).

(d)   N,N'-Bis[1,4,7-tris-(tert-butoxycarbonyl-
methyl)-1,4,7,10-tetraazacyclododecan-10-yl-methyl-
carbonyl]-piperazine

To a solution of the hydrobromide salt of Example 1(b)
(10.0 g, 16.8 mmol) in CHCl₃ (20 mL) and THF (80 mL) was
added 1,1,3,3-tetramethylguanidine (TMG, 1.93 g 16.8
mmol). A white solid was produced which was filtered
off, washed with 20% CHCl₃/THF (100 mL) and identified as
TMG•HBr. The filtrate was concentrated to a clear oil

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which was dissolved in N,N-dimethylformamide (DMF, 200 mL) and treated with the bisbromoacetamide of Example 1(c) (2.62 g , 8.0 mmol) and TMG (1.93 g). The light yellow solution was warmed to 60°C and was allowed to stir for 16 hours under nitrogen. The reaction mixture was cooled to ambient temperature and the DMF was removed under vacuum. The residue was taken up in CH_2Cl_2 (300 mL) and was washed with 1 M Na_2CO_3 (3 x 60 mL). The combined aqueous layer was back-extracted with CH_2Cl_2 (50 mL). The combined CH_2Cl_2 layers were extracted with 1 M HCl (2 x 80 mL) followed by deionized H_2O (2 x 50 mL). The combined HCl and H_2O layers were washed with CH_2Cl_2 (50 mL). The aqueous layer was combined with CH_2Cl_2 (250 mL) in an Erlenmeyer flask, and the pH was adjusted to 9 - 10 with anhydrous Na_2CO_3. The neutralized mixture was transferred to a separating funnel and the layers were separated. The aqueous layer was extracted with CH_2Cl_2 (2 x 100 mL), and all of the basic CH_2Cl_2 layers were combined and washed with H_2O (2 x 70 mL) . The organic layer was dried (Na_2SO_4), filtered, and concentrated to give 12.3 g of an off-white solid. The solid was triturated with ethyl acetate (50 mL) collected by filtration, washed with ethyl acetate (2 x 20 mL), diethyl ether (30 mL), and dried under vacuum to give 8.40 g (76 %) of a white solid. This material analyzed as the _title dimer_ containing two NaX molecules (where X = Cl or Br). ^{1}H NMR (CD_3OD): δ 1.39 (s, 54 H), 1.9 - 3.5 (br m, 56 H). ^{13}C NMR (CD_3OD): δ 174.5, 174.4, 172.2, 82.8, 82.6, 79.5, 56.7, 56.3, 54.0 (br), 49.0 (br), 45.2, 44.9, 42.9, 42.6, 28.5, 28.4. MS (FAB): m/e 1218 (MNa^+). Anal: Calculated (found) for $C_{60}H_{110}N_{10}O_{14}Na_2Cl_2 \cdot 4H_2O$: C, 52.04 (52.14); H, 8.59 (8.68); N, 10.12 (10.07); Na, 3.32 (3.44); Cl, 5.12 (5.16).

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(e)   N,N'-Bis[1,4,7-tris-(carboxymethyl)-1,4,7,10-
tetraazacyclododecan-10-yl-methylcarbonyl]-piperazine

A solution of the dimer of Example 1(d) (10.0 g, 7.2
mol) in CH_2Cl_2 (120 mL) and trifluoroacetic acid (TFA, 80
mL) was allowed to stir at ambient temperature for 1
hour. The volatiles were removed by rotary evaporation
to give a thick brown oil. The residue was redissolved
in CH_2Cl_2 and trifluoroacetic acid as above for 1 hour.
The process had to be repeated seven times to completely
remove all of the t-butyl groups. After the final
treatment with TFA, the crude product was concentrated,
dissolved in H_2O (100 mL) and reconcentrated by rotary
evaporation. The H_2O chase was repeated several times.
The product was precipitated as a white solid by
dissolving the crude mixture in H_2O (20 mL), warming the
solution to 50°C and adding 2-propanol (150 mL) slowly.
The solid was collected by filtration and washed with
2-propanol (2 x 50 mL) and acetone (2 x 30 mL). A
second crop of product was collected from the filtrate
and isolated in a similar fashion. The combined solids
were dried under vacuum to give 7.48 g of material that
contained traces of sodium and TFA. A portion of the
solid, (5.24 g) was dissolved in H_2O (10 mL) and the pH
was adjusted to 10.9 with 2 N NaOH. The solution was
loaded onto a column containing Bio-Rad AG1-X8 (acetate
form) and the column was washed with H_2O (2 L). The
product was then eluted with 0.1 N acetic acid. After
combining desired fractions and concentrating, the
product was precipitated by dissolving in H_2O (50-60°C)
and slowly adding 2:1 acetone/ethanol (60 mL). The
solid was collected, washed with 2:1 acetone/ethanol (60
mL), and dried in vacuo to give 3.31 g (50 %) of the
title product. ${}^1H$ NMR (NaOD, D_2O): δ 2.20 (br s, 16 H),
2.46 (br s, 16 H), 2.88 (s, 12 H), 3.26 (d, 4 H), 3.40
(s, 8 H). ${}^{13}C$ NMR (NaOD, D_2O): δ 180.7, 180.6, 172.4,
59.3, 59.2, 54.9, 54.6, 51.2 (br), 44.6, 44.5, 41.9,

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41.8. MS (FAB): m/e 859.5 (MH+). Anal: Calculated
(found) for $C_{36}H_{62}N_{10}O_{14} \bullet 4.5H_2O$: C, 46.00 (46.00); H, 7.61
(7.45); N, 14.90 (15.01).

(f)   Bisgadolinium complex of N,N'-Bis[1,4,7-tris-
(carboxymethyl)-1,4,7,10-tetraazacyclododecan-
10-yl-methylcarbonyl]-piperazine

To a suspension of the dimeric chelant of Example 1(e)
(5.65 g, 6.0 mmol) in deionized H_2O (120 mL) was added
gadolinium acetate (4.73 g). A clear slightly yellow
solution formed within minutes. The mixture was stirred
at ambient temperature for 3 hours and was then
concentrated by rotary evaporation (50°C) to drive off
acetic acid. The residue was redissolved in H_2O (150 mL)
and the solution was warmed to 40°C. No gadolinium was
detected after 2 hours by a xylenol orange test.
Gadolinium acetate was added in 12.2 mg (0.5 mol%)
increments until a positive test for free gadolinium was
observed. The solution was then treated with ligand to
adjust the titer to ≤0.1 mol% excess ligand. The
complex was precipitated by dissolving in H_2O (40°C) and
adding a 2:1 acetone/ethanol solution. The solid was
collected by filtration, washed with 2:1 acetone/ethanol
(2 x 25 mL) and dried under vacuum at 35°C to give 6.4 g
(80 %) of the title product. MS (FAB): m/e 1168 (MH+).
Anal: Calculated (found) for $C_{36}H_{56}N_{10}O_{14}Gd_2 \bullet 8.75H_2O$: C,
32.64 (32.48); H, 5.58 (5.16); N, 10.57 (10.42); Gd,
23.73 (23.31).

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Example 2

Synthesis of N,N'-bis[1,4,7-tris-(carboxymethyl)-
1,4,7,10-tetraazacyclododecan-10-yl-methylcarbonyl]-
N,N'-dimethylethylenediamine and the gadolinium complex
thereof

(a)   N,N'-Dimethylethylenediamine
bis(bromoacetamide)

To a 1 L three-necked flask equipped with a magnetic
stir bar, reflux condenser and addition funnel, was
added bromoacetyl bromide (46.95 g , 232.6 mmole) and
CHCl$_3$ (100 mL). The addition funnel was charged with a
solution of N,N'-dimethylethylenediamine (10.0 g, 113.4
mmol) and triethylamine (39.5 mL) in CHCl$_3$ (100 mL). The
flask was chilled to -15°C by means of an ethylene
glycol/CO$_2$ bath, and the amine was slowly added to the
acid bromide. After the addition was complete, the
mixture was allowed to warm to ambient temperature and
stirred for 1 hour. The flask was then cooled to 0°C
and H$_2$O (50 mL) was slowly added. The mixture was
diluted with CHCl$_3$ (250 mL) and the layers were
separated. The organic layer was extracted with H$_2$O (3 x
50 mL), 0.05 N NaOH (3 x 50 mL), and H$_2$O (2 x 50 mL), and
was dried (Na$_2$SO$_4$). The solution was filtered and
concentrated. The material was methanol purified by
filtration through a bed of silica gel. The product
eluted with 2 - 5% methanol/CH$_2$Cl$_2$. After combining and
concentrating the desired fractions, 14.95g (39.9%) of a
beige solid was obtained. The title product was
recrystallized from warm 2-propanol affording 11.94 g.
^{1}H NMR (CDCl$_3$): δ [2.99 (s), 3.11 (s), 3.13 (s); 6 H],
[3.53 (s), 3.57 (s); 4 H], 3.81 (s), 3.84 (s), 3.91 (s);
4 H].

(b)   N,N'-Bis[1,4,7-tris-(tert-
butoxycarbonylmethyl)1,4,7,10-tetraazacyclododecan-10-
yl-methylcarbonyl]-N,N'-dimethylethylenediamine

To a solution of the hydrobromide salt of Example 1(b)
(8.0 g, 13.4 mmol) in CHCl₃ (20 mL) and THF (80 mL) was
added 1,1,3,3-tetramethylguanidine (TMG, 1.547 g, 13.4
mmol)). A white solid was produced which was filtered
off, washed with 20% CHCl₃/THF (100 mL) and identified as
TMG•HBr. The filtrate was concentrated to a clear oil
which was dissolved in N,N-dimethylformamide (DMF, 200
mL) and treated with the bis(bromoacetamide) of Example
2(a) (2.21 g, 6.7 mmol) and TMG (1.547 g). The light
yellow solution was warmed to 60°C and was allowed to
stir for 16 hours under nitrogen. The reaction mixture
was cooled to ambient temperature and the DMF was
removed under vacuum. The residue was taken up in CHCl₃
(200 mL) and washed with 1 M Na₂CO₃ (3 x 40 mL). The
combined aqueous layer was back-extracted with CHCl₃ (50
mL). The combined CHCl₃ layers were extracted with 0.8 M
HCl (2 x 50 mL) followed by deionized H₂O (2 x 50 mL).
The combined HCl and H₂O layers were washed with CHCl₃
(50 mL). The aqueous layer was combined with CHCl₃ (200
mL) in an Erlenmeyer flask, and the pH was adjusted to
between 9.5 with Na₂CO₃. The neutralized mixture was
transferred to a separating funnel and the layers were
separated. The aqueous layer was extracted with CHCl₃ (2
x 100 mL) , and the basic CHCl₃ layers were combined and
washed with H₂O (2 x 50 mL). The organic layer was dried
over anhydrous Na₂SO₄, filtered and concentrated to give
7.50 g (93 %) of the title product as a yellow oil. ¹H
NMR (CDCl₃): δ 1.24 (s, 54 H), 1.90-3.39 (br m, 67 H).
¹³C NMR (CDCl₃): δ 27.7, 27.8, 34.9. 45.7, 48.3 (br),
52.0 (br), 55.4, 55.6, 56.3, 81.4, 81.6, 81.6, 81.7,
81.8, 171.5, 172.6, 172.7.

(c)   N,N'-Bis[1,4,7-tris-(carboxymethyl)-1,4,7,10
tetraazacyclododecan-10-yl-methylcarbonyl]-N,N-dimethyl-
ethylenediamine

A solution of the dimer of Example 2(b) (7.50 g) in
CH_2Cl_2 (100 mL) and trifluoroacetic acid (TFA, 60 mL) was
allowed to stir at ambient temperature for 1 hour. The
volatiles were removed by rotary evaporation to give a
thick brown oil. The residue was redissolved in CH_2Cl_2
(20 mL) and trifluoroacetic acid (15 mL) as above for 1
hour. The process was repeated seven times to
completely remove all of the t-butyl groups. After the
final treatment with TFA, the crude product was
concentrated, dissolved in H_2O (100 mL) and
reconcentrated by rotary evaporation. The H_2O chase was
repeated several times. The product was purified by
ion-exchange chromatography (Bio Rad AG1-X8, acetate
form) using 0.1 N acetic acid to elute the product.
Desired fractions were combined and concentrated to give
2.36 g (37 %) of the title product as an off white solid
after lyophilization. ^1H NMR (NaOD, D_2O): δ 2.0-3.7
(br, m). ^{13}C NMR (NaOD, D_2O): δ 34.5, 35.4 (br), 43.7,
45.6, 47.2, 47.2, 48.9, 49.6, 50.0, 51.6 (br), 52.5,
55.1, 56.0, 57.3, 59.1, 63.3, 173.4 (br), 179.2, 180.5,
181.4. MS (FAB): m/e 861 (MH$^+$), 883 (MNa$^+$), 917 (M +
NaCl - H)$^+$], 973 [(M + 2NaCl -2H)$^+$].

(d)   Bisgadolinium complex of N,N'-Bis[1,4,7-tris-
(carboxymethyl)-1,4,7,10-tetraazacyclododecan-10-yl-
methylcarbonyl]-N,N-dimethyl-ethylenediamine

To a solution of the dimeric chelant of Example 2(c)
(1.88 g, 2.0 mmol) in deionized H_2O (40 mL) was added
gadolinium acetate (1.53 g). The light yellow solution
was stirred at 40°C for 1 hour and was then concentrated
by rotary evaporation (50°C) to drive off acetic acid.

The residue was redissolved in H_2O (100 mL) and the
solution was warmed to 40°C. The reaction was allowed
to stir overnight at ambient temperature. Gadolinium
acetate was added in 0.01 mmol increments until a
positive test for free gadolinium was observed. The
solution was then treated with ligand to adjust the
titer to 0.1 mol% excess ligand. The solution was
concentrated to dryness and was triturated with 2:1
diethylether/$CHCl_3$ to give the title product as a beige
solid (2.57 g, 98 %).

Example 3

Synthesis of N,N'-Bis[1,4,7-tris-(carboxymethyl)-
1,4,7,10-tetraazacyclododecan-10-yl-
methylcarbonyl]tetrahydroquinoxaline and the gadolinium
complex thereof

(a)   Tetrahydroquinoxaline bis(chloroacetamide)

To a 1 L three-necked round bottomed flask equipped with
a magnetic stir bar, reflux condenser and addition
funnel, was added chloroacetyl chloride (9.6 mL, 0.120
mol) and $CHCl_3$ (100 mL). The addition funnel was charged
with a solution of tetrahydroquinoxaline (8.0 g, 0.0596
mol) and triethylamine (20.8 mL) in $CHCl_3$ (100 mL). The
flask was chilled to 0°C, and the amine was slowly added
to the acid chloride. After the addition was complete,
the mixture was allowed to warm to ambient temperature
and stir for 1 hour. The flask was then cooled to 0°C
and H_2O (50 mL) was slowly added. The mixture was
diluted with $CHCl_3$ (250 mL) and the layers were
separated. The organic layer was washed with H_2O (50
mL), 0.05 N NaOH (2 x 50 mL), H_2O (50 mL), 1 N HCl (3 x

50 mL) and H_2O (2 x 50 mL), and was dried over anhydrous Na_2SO_4. The solution was filtered and concentrated to a brown solid. The solid was collected by filtration, washed with 2-propanol (2 x 30 mL) and diethylether (2 x 40 mL) and dried to yield the title product (4.41 g, 51.5%). ^1H NMR (DMSO-d6): δ 3.89 (s, 4 H), 4.01 (s, 4 H), 7.25 (s, 2 H), 7.65 (br s, 2 H).

(b) N,N'-Bis[1,4,7-tris-(tert-butoxycarbonyl-methyl)-1,4,7,10-tetraazacyclododecan-10-yl-methyl-carbonyl]tetrahydroquinoxaline

To a solution of the hydrobromide salt of Example 1(b) (8.0 g, 13.4 mmol) in $CHCl_3$ (20 mL) and THF (80 mL) was added 1,1,3,3-tetramethylguanidine (TMG, 1.547 g, 13.4 mmol)). A white solid was produced which was filtered off, washed with 20% $CHCl_3$/THF (100 mL) and identified as TMG•HBr. The filtrate was concentrated to a clear oil which was dissolved in N,N-dimethylformamide (DMF, 250 mL) and treated with bis(chloroacetamide) of Example 3(a) (1.928 g, 6.72 mmol) and TMG (1.547 g). The light yellow solution was warmed to 60°C and was allowed to stir for approximately 16 hours under nitrogen. The reaction mixture was cooled to ambient temperature and the DMF was removed under vacuum. The residue was taken up in $CHCl_3$ (200 mL) and was washed with 1 M Na_2CO_3 (3 x 40 mL). The combined aqueous layer was back-extracted with $CHCl_3$ (50 mL). The combined $CHCl_3$ layers were extracted with 1 M HCl (2 x 50 mL) followed by deionized H_2O (2 x 50 mL). The combined HCl and H_2O layers were washed with $CHCl_3$ (2 x 50 mL). The aqueous layer was combined with $CHCl_3$ (250 mL) in an Erlenmeyer flask, and the pH was adjusted to 9.5 (Na_2CO_4). The neutralized mixture was transferred to a separating funnel and the layers were separated. The aqueous layer was extracted with $CHCl_3$ (2 x 50 mL), and all $CHCl_3$

layers were combined and washed with brine (2 x 40 mL).
The organic layer was dried (Na_2SO_4), filtered, and
concentrated to give 10.60 g of a yellow oil. The title
product was purified further by passing through a bed of
silica gel and eluting the product with 10%
methanol/$CHCl_3$ to give 7.06 g (84.6 %). ^{1}H NMR ($CDCl_3$):
δ 1.30 (s), 1.38 (s), 1.41 (s), 2.0-3.7 (br m), 7.28
(s).

(c)   N,N'-Bis[1,4,7-tris-(carboxymethyl)-1,4,7,10-
tetraazacyclododecan-10-yl-methylcarbonyl]tetra-
hyquinoxaline

A solution of the dimer of Example 3(b) (7.06 g) in
CH_2Cl_2 (70 mL) and trifluoroacetic acid (TFA, 60 mL) was
allowed to stir at ambient temperature for 1 hour. The
volatiles were removed by rotary evaporation to give a
thick brown oil. The residue was redissolved in CH_2Cl_2
(70 mL) and trifluoroacetic acid (60 mL) as above for 1
hour. The process was repeated nine times to completely
remove all of the t-butyl groups. After the final
treatment with TFA, the crude product was concentrated,
dissolved in H_2O (100 mL), and reconcentrated by rotary
evaporation. The H_2O chase was repeated several times.
The product was purified by ion-exchange chromatography
column (Bio Rad AG1-X8, acetate form) using 0.1 N acetic
acid to elute the product. Desired fractions were
combined and concentrated to give 3.26 g of an off white
solid after lyophilization The solid was triturated
with ethanol (50 mL), collected by filtration, washed
with diethyl ether and dried to yield the title product
(3.18 g, 48 %). ^{1}H NMR (NaOD, D_2O): δ [2.88 (br s),
3.19 (br s), 3.43 (s), 3.53-3.68 (m); 56 H], 7.07 (m, 3
H), 7.59 (br s, 1 H).

(d)   Bisgadolinium complex of N,N'-bis[1,4,7-tris
(carboxymethyl)-1,4,7,10-tetraazacyclododecan-10-yl-
methylcarbonyl]-tetrahyquinoxaline

To a solution of the dimeric chelant of Example 3(c)
(2.50 g, 2.53 mmol) in 100 mL deionized H_2O (100 mL) was
added gadolinium acetate (1.9827 g, 96% of
theroretical). The clear solution was stirred at 40°C
for 1 hour and was then concentrated by rotary
evaporation (50°C) to drive off acetic acid. The
residue was redissolved in H_2O (100 mL) and the solution
was warmed to 40°C and the reaction allowed to stir
overnight at ambient temperature. Gadolinium acetate
was added in 0.5 mol% increments until a positive test
for gadolinium was observed. The solution was then
treated with ligand to adjust the titer to 0.5 mol%
excess ligand. The solution was lyophlized to give 3.28
g of the title product as an off-white solid. Mass
spectrum (FAB): m/e 1217 (MH$^+$).

Example 4

Synthesis of N,N'-Bis[1,4,7-tris-(carboxymethyl)-
1,4,7,10-tetraazacyclododecan-10-yl-
methylcarbonyl]ethylenediamine and the gadolinium
complex thereof

(a)   Ethylenediamine bis(chloroacetamide)

To a solution of ethylenediamine (3.0 g, 0.05 mol) in
$CHCl_3$ (50 mL) cooled to -15°C was added chloroacetyl
chloride (7.96 mL, 0.10 mol) in $CHCl_3$ (40 mL) dropwise.
A white precipitate formed and the reaction mixture was
stirred overnight at ambient temperature. After 16

hours the reaction mixture was filtered and the white
solid was washed with $CHCl_3$ (30 mL) followed by water
(100 mL). The $CHCl_3$ layer was washed with water (2 x 25
mL), dried (Na_2SO_4), and filtered. After being dried
under vacuum, the white solid was recrystallized from
ethanol and filtered hot to give a clear filtrate from
which the product crystallized upon standing. The solid
was collected by filtration and washed with isopropyl
alcohol (20 mL). The mother liquor from the
recrystallization and the $CHCl_3$ layer were combined and
concentrated yielding a white solid which was
recrystallized from ethanol and then washed with
isopropyl alcohol. The title product (4.9 g, 46%) was
obtained as a white solid. ^1H NMR ($CDCl_3$) δ 7.05 (br, 2
H), 4.04 (s, 4 H), 3.49 (s, 4 H). ^{13}C NMR (DMSO-d_6) δ
166.2, 42.7, 38.5.

(b)   N,N'-Bis[1,4,7-tris-(tert-butoxycarbonyl-methyl)-1,4,7,10-tetraazacyclododecan-10-yl-methylcarbonyl-ethylenediamine

The hydrobromide salt of Example 1(b) (4.7 g, 7.9 mmol)
was dissolved in 20% $CHCl_3$/THF (50 mL). To this solution
was added TMG (0.99 mL, 7.9 mmol) with the solution
quickly becoming cloudy as TMG·HBr precipitated. After
stirring for 15 minutes, the solution was filtered and
the filtrate concentrated to a yellow oil. The oil was
taken up in THF (80 mL) and transfered to a three necked
round bottom flask. The flask was then charged with NaI
(590 mg, 3.9 mmol), TMG (0.99 mL, 7.9 mmol), and finally
the bischloroacetamide of Example 4(a) (837 mg, 3.93
mmol). The reaction mixture was placed under N_2 and
heated to 68°C. After 16 hours, the reaction mixture
was filtered and the filtrate concentrated to a gummy
solid. The residue was taken up in CH_2Cl_2 and washed
with 1.0 N Na_2CO_3 (3 x 50 mL). The aqueous phase was

back extracted with CH_2Cl_2. The organic portions were
combined and extracted with 1 M HCl (1 x 80 mL). The
aqueous phase was then washed with CH_2Cl_2 and the pH of
the aqueous phase was adjusted to 9 with Na_2CO_3. The
aqueous layer was then extracted with CH_2Cl_2 (1 x 200 mL,
2 x 100 mL) and the CH_2Cl_2 layer washed with water (2 x
75 mL), dried ($MgSO_4$), and filtered. Concentration and
drying under vacuum yielded 3.6 grams (79%) of the title
compound as a pale yellow solid. ^1H NMR ($CDCl_3$) δ 3.34 -
2.00 (br band, 57 H), 1.44 (br s, 54 H).

(c)   N,N'-Bis[1,4,7-tris-(carboxymethyl)-1,4,7,10-
tetraazacyclododecan-10-yl-methylcarbonyl]-ethylene-
diamine

The ethylenediamine dimer of Example 4(b) (6.7 g; 5.7
mmol) was dissolved in CH_2Cl_2 (40 mL) and a 1:1 solution
of TFA/CH_2Cl_2 (20 mL) was added. The resulting mixture
was stirred for one hour and then concentrated. This
procedure was repeated eight times to complete the
deprotection. After the final deprotection, the
solution was concentrated and chased with water (3 x 25
mL) to yield a 6.2 grams of a brownish solid. The solid
was dissolved in water (15 mL), the pH was adjusted to
10.9 with 3 N LiOH, and the solution was loaded onto
AG1-X8 ion exchange resin (acetate form). The column
bed was washed with water (1 L), and the dimer eluted
from the column with 0.1 N acetic acid with the desired
fractions being combined and concentrated yielding 2.5 g
(51%) of the title product as a pale yellow solid. ^1H
NMR (D_2O) δ 3.62 - 2.89 (br band).

(d)   Bisgadolinium complex of N,N'-bis[1,4,7-tris-
(carboxymethyl)-1,4,7,10-tetraazacyclododecan-10-yl-
methylcarbonyl]ethylenediamine

The dimeric chelant of Example 4(c) (2.12 g, 2.32 mmol)
was dissolved in water (50 mL) and gadolinium acetate
(1.81 g, 2.25 mmol) was added. The mixture was allowed
to stir at ambient temperature for 1.5 hours, the
solution was concentrated, and the residue was taken up
in water (50 mL). After another 1.5 hours, the
solution gave a positive xylenol orange test, therefore
the solution was concentrated and chased with water (1x
20 mL). The residue was taken up in water (50 mL) and
allowed to stir overnight at ambient temperature.
After stirring for 16 hours, the solution was chased
with water to remove the acetic acid formed.
Additional ligand was added in 21 mg (0.07 mmol)
portions until the xylenol orange test gave a positive
(purple) color. A final portion of 21 mg of ligand was
added and the solution was stirred for two hours at
40°C. A xylenol orange test was performed and gave a
negative result. The solution was then concentrated to
give a pale yellow solid. The solid was dissolved in
warm water (15 mL) to which was added a 2:1
acetone/ethanol solution (85 mL) from which a white
solid precipitated. The solid was collected by
filtration and washed with 2:1 acetone/ethanol solution
(200 mL) followed by acetone (30 mL) and dried in a
vacuum oven (35°C) for 3.5 hours to yield 2.4 g of the
title product as a white solid. MS (FAB): m/e 1143.2
(MH+). Anal: Calculated for $C_{34}H_{54}Gd_2N_{10}O_{14} \cdot 3.4H_2O$:
C,29.53; H,5.89; Gd,22.74; N,10.13. Found C,29.47;
H,5.59; Gd,22.54; N,9.81.

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Example 5

Synthesis of N,N'-bis[1,4,7-tris-(carboxymethyl)-
1,4,7,10-tetraazacyclododecan-10-yl-
methylcarbonyl]homopiperazine and the gadolinium complex
thereof

(a)   Homopiperazine bis(chloroacetamide)

Homopiperazine (10 g; 100 mmol) was dissolved in
chloroform (150 mL) and triethylamine (28 mL; 200 mmol)
was added to the mixture. The solution was cooled to -
30°C under nitrogen and chloroacetyl chloride (17 mL, 213
mmol) was added over a period of 1 hour. The solution
was stirred at -30°C for 1hour, at 0°C for 2 hour and at
ambient temperature overnight.. The solution was washed
with water (4 x 50 mL), dried over anhydrous MgSO₄,
filtered and concentrated. The oily residue was
purified by column chromatography on silica gel (5 %
methanol in chloroform) to yield the title product as an
oil which solidified on prolonged standing (10.3 g; 41
%). ¹H NMR (CDCl₃) : 4.04 (m, 4 H), 3.62 (m, 8 H), and
1.94 (m, 2 H).

(b)   N,N'-Bis[1,4,7-tris-(tert-
butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecan-10-
yl-methylcarbonyl]homopiperazine

A solution of the bis(chloroacetamide) of Example 5(a)
(1.7 g, 6.72 mmol),   DO3A-tri-t-butyl ester (8 g, 13.43
mmol) and tetramethylguanidine (2.6 mL, 20.75 mmol) in
acetonitrile (300 mL) was heated at 60°C for 25 hours
under nitrogen. The solvent was removed under reduced

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pressure and the residue redissolved in chloroform (60
mL). The solution was washed with 1 M sodium carbonate
(2 x 50 mL) and water (2 x 50 mL) and extracted with 1 M
HCl (4 x 50 mL) followed by water (2 x 50 mL). The
aqueous extracts were combined and basified with solid
sodium carbonate. The crude product that separated as
an oil was extracted with chloroform (3 x 100 mL), dried
over anhydrous magnesium sulfate, filtered, and
concentrated to yield the crude title product as a
yellow solid (9.77 g). ^{1}H NMR (CDCl$_3$) : 3.44-2.45 (m,
56 H), 1.84 (s, 2 H), 1.42 (s, 18 H), 1.40 (s, 36 H).

(c)   N,N'-Bis[1,4,7-tris-(carboxymethyl)-1,4,7,10-
tetraazacyclododecan-10-yl-methylcarbonyl]homopiperazine

A solution of the crude t-butyl ester of Example 5(b)
(9.77 g) in CH$_2$Cl$_2$ (30 mL) was treated with
trifluoroacetic acid (30 mL) and the mixture stirred at
ambient temperature for 1 hour. The solution was
concentrated and the process repeated seven times. The
oily residue (10.5 g) obtained after the final
deprotection was dissolved in acetone (30 mL) and was
precipitated with chloroform. Filtration and drying
under vacuum yielded a yellow solid (8.33 g). The solid
was dissolved in water (20 mL) and the pH of the
solution was adjusted to 11.0 with 3.0 N LiOH. The
solution was loaded onto an AG1-X8 ion exchange column
(acetate form) and the column bed was washed with water
(1 L). The desired dimer was eluted with 0.1 N acetic
acid with the desired fractions being combined and and
concentrated. Lyophilzation of the product yielded 3.8
g (54 %) of the title product as a yellow solid. ^{1}H NMR
(D$_2$O) δ 3.63 - 2.74 (br band, 56 H), 1.6 (br s, 2 H)

(d)   Bisgadolinium complex of N,N'-bis[1,4,7-tris-
(carboxymethyl)-1,4,7,10-tetraazacyclododecan-10-yl-
methylcarbonyl]homopiperazine

To the dimeric chelant of Example 5(c) (3.11g, 3.26
mmol) dissolved in water (60 mL) was added gadolinium
acetate (2.52 g, 6.30 mmol). The solution was warmed to
40°C for one hour and then concentrated to remove the
acetic acid formed. Water (60 mL) was again added, the
solution was stirred for one hour, and the process to
remove the acetic acid repeated. The solid was then
taken up in water (60 mL) and stirred overnight at
ambient temperature. The solution gave a weakly
positive xylenol orange test, therefore ligand (31 mg,
0.07 mmol) was added. After 1.5 hours at 40°C the
reaction mixture gave a negative xylenol orange test.
The solution was concentrated and then dissolved in
ethanol. Acetone was added and the precipitated complex
collected by filtration. The solid was taken up in
acetone and stirred for 1 hour and then collected by
filtration. The solid was dried in a vacuum oven (35°C)
for two hours to give 3.2 g (76 %) of the title product
as a fine white powder. MS (FAB): m/e 1183.3 (MH$^+$).
Anal: Calculated for $C_{37}H_{58}Gd_2N_{10}O_{14} \cdot 12.3 \; H_2O \cdot 0.72$ acetone:
C,32.55; H,6.06; Gd,21.77; N,9.69. Found C,32.92;
H,5.69; Gd,21.47; N,9.91.

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Example 6

Synthesis of N,N'-bis[1,4,7-tris-(carboxymethyl)-
1,4,7,10-tetraazacyclododecan-10-yl-methylcarbonyl]-
2,2'-(ethylenedioxy)diethylamine and the gadolinium
complex thereof

(a)   2,2'-(Ethylenedioxy)diethylamine
bis(chloroacetamide)

To a solution of 2,2'(ethylenedioxy)diethylamine (10.0
g, 0.0675 mol) in CH_2Cl_2 (190 mL) was added Na_2CO_3 (14.2
g, 0.135 mol). The resulting mixture was chilled in an
ice bath and placed under a stream of N_2 to which was
added chloroacetyl chloride (10.7 mL, 0.135 mol) in
CH_2Cl_2 dropwise over a period of 25 minutes. Upon
completion of addition, the ice bath was removed and the
mixture was stirred at ambient temperature for two
hours. The reaction mixture was then filtered and the
filtrate was washed with water (150 mL), saturated $NaHCO_3$
(150 mL), and finally water (150 mL). The organic phase
was dried (over Mg_2SO_4), filtered, and concentrated to
yield a yellow oil which solidified when placed under
vacuum. The solid was triturated with a 1:1 mixture of
ethyl acetate/hexane (150 mL). A white solid was
collected by filtration and dried in a vacuum oven
(50°C) for 6 hours yielding 6.9 g (34%) of the title
product. ^{1}H NMR ($CDCl_3$) δ 6.97 (br s, 2 H), 4.03 (s, 4
H), 3.60 (s, 4 H), 3.56 (distorted t, 4 H), 3.50 (t, 4
H); ^{13}C NMR (CDCl) δ 165.9, 70.1, 69.1 42.4, 39.3.

<u>(b)</u>   <u>N,N'-Bis[1,4,7-tris-(tert-</u>
<u>butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecan-10-</u>
<u>yl-methylcarbonyl]-2,2'-(ethylenedioxy)diethylamine</u>

The hydrobromide salt of Example 1(b) (19.8 g, 33.2
mmol) was dissolved in 20% $CHCl_3$/THF (250 mL). To this
solution was added TMG (4.16 mL, 33.2 mmol) and the
solution quickly became cloudy as TMG·HBr precipitated.
After stirring for 20 minutes, the solution was filtered
with the filtrate being concentrated to a thick yellow
oil. The residue was taken up in THF (100 mL) and
transferred to a 1 L flask which was then charged with
NaI (2.48 g, 16.6 mmol), TMG (4.16 ml, 33.2 mmol), and
finally the bis(chloroacetamide) of Example 6(a) (5.0 g,
16.6 mmol). Additional THF (150 mL) was added, the
system was placed under a stream of N_2, and the reaction
mixture was heated to 65°C. After four days, the
reaction mixture was filtered and the filtrate
concentrated to a reddish solid. The solid was taken up
in CH_2Cl_2 (110 mL) and washed with water (3 x 100 mL).
The organic portion was dried ($MgSO_4$), filtered and
concentrated to yield 22.14 g (114%) of the <u>title</u>
<u>product</u> as a tacky yellow solid which was not analyzed
for salts. ^{1}H NMR (CDCl$_3$) δ 3.39-2.06 (br band, 62 H),
1.25 (br s, 54 H).

<u>(c)</u>   <u>N,N'-Bis[1,4,7-tris-(carboxymethyl)-1,4,7,10-</u>
<u>tetraazacyclododecan-10-yl-methylcarbonyl]-2,2'-</u>
<u>(ethylenedioxy)diethylamine</u>

The crude dimer of Example 6(b) (22.0 g, 18.7 mmol) was
dissolved in CH_2Cl_2 (25 mL). To this solution was added
a mixture of TFA (25 mL) and CH_2Cl_2 (10 mL). After
stirring for 2 hours at ambient temperature, the
reaction was concentrated. This procedure was repeated
seven times to complete the deprotection. After the

final deprotection, the solution was concentrated and
chased with water (3 x 40 mL). The reddish semi-solid
was then dissolved in water (30 mL) and the pH adjusted
to 2.3 with 3.0 N NH$_4$OH. The solution was loaded onto an
AG50-X8 ion-exchange resin (H$^+$ form) and the column bed
washed with water (1.8 L). The dimer was eluted from
the column with 0.5 N NH$_4$OH with the desired fractions
being combined and concentrated to yield 9.8 g (57 %) of
the title product as a yellow solid. ^{1}H NMR (D$_2$O) δ
3.61-2.92 (br band). MS (FAB): m/e 921.4 (MH$^+$).

(d)   Bisgadolinium complex of N,N'-bis[1,4,7-tris-
(carboxymethyl)-1,4,7,10-tetraazacyclododecan-10-yl-
methylcarbonyl]-2,2'-(ethylenedioxy)diethylamine

To a solution of the DO3A dimer of Example 6(c) (5.90 g,
5.94 mmol) in water (50 mL) was added gadolinium acetate
(3.61 g, 8.91 mmol) and the reaction mixture was warmed
to 40°C for 1.5 hours at which time the xylenol orange
test was negative. Gadolinium acetate (240 mg, 0.594
mmol) was added in increments until a positive (purple)
xylenol orange was achieved. Ligand was then added to
achieve a negative xylenol orange test. The solution
was reduced in volume and lyophilized yielding 7.58 g of
crude complex. The crude complex was purified by HPLC
employing a reverse phase C18 column using 2%
methanol/water as the mobile phase. MS (FAB): m/e
1231.0 (MH$^+$) Anal: Calculated for C$_{38}$H$_{62}$Gd$_2$N$_{10}$O$_{16}$•7.65H$_2$O:
C,33.38; H,5.70; Gd,23.00; N,10.24. Found C,33.48;
H,5.79; Gd,22.75; N,10.50.

Example 7

Synthesis of N,N'-bis[1,4,7-tris-(carboxymethyl)-
1,4,7,10-tetraazacyclododecan-10-yl-methylcarbonyl]-
N,N'-bis(2,3-dihydroxypropyl)-ethylenediamine and the
gadolinium complex thereof

(a)   1,2,5,6-Di-isopropylidene-D-mannitol

To a solution of (411 g, 3.01 mol) of $ZnCl_2$ in 1.5 L of
acetone cooled to 0°C was added (250 g, 1.37 mol) of
D-mannitol and the mixture stirred at ambient
temperature for 5 hours. The solid was filtered off and
the filtrate poured into a solution of diethyl ether
(1.8 L) and potassium carbonate (470 g, 3.4 mol)
producing vigorous bubbling. The solution was stirred
for 1 hour. A white precipitate was filtered off and
the filtrate was dried (K_2CO_3), and concentrated leaving
a white solid which was recrystallized from n-butyl
ether to give 160 g (50%) of the title product as white
needles. ^1H NMR ($CDCl_3$) δ 4.2 (m, 4 H), 4.0 (t, 2 H),
3.7 (d, 2 H), 2.7 (s, 2 H), 1.4 (d, 12 H).

(b)   Isopropylidene-glyceraldehyde

The mannitol derivative of Example 7(a) (81 g, 0.311
mol) was suspended in CH_2Cl_2 (1.1 L) and saturated $NaHCO_3$
(40 mL) and stirred at ambient temperature. Sodium
periodate (100 g, 0.47 mol) was added in four portions
over 20 minutes and the solution was stirred vigorously
at 0°C for 3 hours. The solvent was decanted off and the
remaining solid was stirred in CH_2Cl_2 and then filtered
to remove the excess solid. The two organic portions
were combined and concentrated in vacuo. The resulting

oil was distilled at reduced pressure to give the title
product as a clear, viscous oil. Yield (59 g, 75%). ^{1}H
NMR (CDCl$_3$) δ 4.3 (t, 1 H), 3.9 (m, 2 H), 1.4 (d, 6 H).

(c)   N,N'-Bis(2,3-dihydroxypropyl)ethylenediamine
bisacetonide

Ethylenediamine (13.2 g, 0.22 mol) was dissolved in
methanol (150 mL) and the solution was adjusted with a
1:1 HCl/methanol mixture to pH 7. The solution was
cooled with an ice bath and
isopropylidene-glyceraldehyde of Example 7(b) (59 g,
0.45 mol) was added followed by portionwise addition of
NaCNBH$_3$ (28.3g, 0.45 mol). The reaction mixture was
stirred at 25°C under N$_2$ for 72 hours. The pH of the
solution was then lowered to 3 with HCl/methanol and the
solvent was stripped off in vacuo. The solid was taken
up in H$_2$O and extracted with diethyl ether (3 x 200 mL).
The pH of the aqueous phase was raised to 11 and
extracted again with diethyl ether (4 x 200 mL). The
ether washes were combined, dried (MgSO$_4$) and
concentrated to a yellow oil. The title product was
isolated by flash chromatography. (5% methanol/CHCl$_3$).
Yield (37 g, 58%). ^{1}H NMR (CDCl$_3$) δ 4.1 (m, 4 H), 3.3
(m, 2 H), 2.6 (m, 8 H), 1.4 (d,12 H).

(d)   N,N'-Bis(2,3-dihydroxypropyl)ethylenediamine
bisacetonide bischloroacetamide

The compound of Example 7(c) (37 g, 0.13 mol) and
triethylamine (25.96 g, 0.25 mol) were combined in
CH$_2$Cl$_2$ (300 mL). Chloroacetyl chloride (28.9 g, 0.25
mol) was added dropwise at 0°C under N$_2$. A color change
was observed along with a white precipitate as the
reaction mixture was allowed to return to ambient

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temperature. After 24 hours, H_2O (150 mL) was added, the organic layer was separated, washed with water (3 x 100 mL), dried ($MgSO_4$) and concentrated to a dark viscous oil. The _title product_ (23 g, 41%) was isolated by flash chromatography, 10% methanol/$CHCl_3$. MS (FAB): m/e 442 (MH$^+$).

(e)   N,N'-Bis[1,4,7-tris-(tert-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecan-10-yl-methylcarbonyl]N,N'-bis(2,3-dihydroxypropyl)-ethylenediamine bisacetonide

The hydrobromide salt of Example 1(b) (12 g, 0.02 mol) and the compound of Example 7(d) (4.5 g, 0.01 mol) were dissolved in CH_3CN (300 mL) and tetramethylguanidine (7.6 mL, 0.61 mol). The reaction mixture was heated to 60°C and stirred under N_2 for 6 days. The solvent was stripped off and the resulting dark oil taken up in $CHCl_3$ and extracted with water (3 x 100 mL), dried ($MgSO_4$) and concentrated to give (12 g, 80%) of the _title product_ MS(FAB): m/e 1398 (MH$^+$).

(f)   N,N'-Bis[1,4,7-tris-(carboxymethyl)-1,4,7,10-tetraazacyclododecan-10-yl-methylcarbonyl]N,N'-bis(2,3-dihydroxypropyl)ethylenediamine

The dimer of Example 7(e) was dissolved in $CHCl_3$ (175 mL) and trifluoroacetic acid (175 mL) was added dropwise. The reaction mixture was allowed to stir for 1 hour at ambient temperature under N_2 and then concentrated in vacuo to a dark oil. The acid treatment was repeated ten times to remove all t-butyl groups and the acetonides. The solvent was stripped off and the _title product_ purified via preparative HPLC. (Supelco activated C18 reverse-phase column, 3% methanol mobile

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phase). Yield (9 g, 85%); MS (FAB): m/e 982 (MH⁺).

(g)   Gadolinium complex of N,N'-bis[1,4,7-tris-
(carboxymethyl)-1,4,7,10-tetraazacyclododecan-10-yl-
methylcarbonyl]-N,N'-bis(2,3-dihydroxypropyl)-
ethylenediamine

The dimeric chelant of Example 7(f) (10 g, 10.2 mmol)
was dissolved in H_2O (175 mL). Gadolinium triacetate
(5.45 g, 0.016 mol, 80% of the theoretical
stoichiometric amount) was added and the pH was adjusted
to 7 using NH_4OH. The reaction mixture was stirred at
50°C for 2 hours. Additional Gadolinium triacetate was
added in 5 mol% increments until a xylenol orange test
for gadolinium was positive. The reaction was stirred
for an additional 24 hours and the xylenol orange test
repeated, giving a positive result. Purification of the
title product was achieved by preparative HPLC (Supelco
activated C18 reverse-phase column, 100% H_2O mobile
phase) to yield (500 mg, 5%) MS (FAB): m/e 1290 (MH⁺)
Anal. calculated for $C_{40}H_{66}Gd_2N_{10}O_{18}$·3 H_2O: C, 35.76; H,
5.4; Gd, 23.36; N, 10.43;   found C, 35.3; H, 5.59; Gd,
23.17; N, 10.9.

Example 8

Synthesis of N,N'-bis[1,4,7-tris-(carboxymethyl)-
1,4,7,10-tetraazacyclododecan-10-yl-methylcarbonyl]-
N,N'-bis(2-hydroxyethyl)ethylenediamine and the
gadolinium complex thereof

(a)   bis(BOC)-bis(2-hydroxyethyl)ethylene diamine

N,N'-bis(hydroxyethyl)ethylene diamine (2 g; 13.5 mmol)
was dissolved in methanol and the solution was treated
with di-t-butyl dicarbonate (6.0 g; 27.5 mmol). The
solution was stirred at ambient temperature for 9 hours
under nitrogen and concentrated. The residue was washed
with petroleum ether and dried under vacuum to obtain
the title product as a colorless solid (4.48 g; 95.3 %).
^{1}H NMR (CDCl$_3$): δ 1.42 (s, 18H), 3.44-3.35 (m, 8H), 3.44
(s), 3.7 (m, 4H), 4.89 (br s, 2H).

(b)   Bis(BOC)-bis(2-benzyloxyethyl)ethylene
diamine

An 80 % mineral oil suspension of sodium hydride (0.75
g; 25 mmol) was washed with tetrahydrofuran under a
blanket of nitrogen and re-suspended in tetrahydrofuran
(25 mL). Benzyl bromide (20 mL; 169 mmol) and the
diamine of Example 8(a) (4.37 g; 12.54 mmol) were added
in succession. Following a vigorous initial reaction,
the suspension was stirred overnight at ambient
temperature under nitrogen. The solution was
concentrated under vacuum and the excess benzyl bromide
was distilled off under vauum at 40°C and the residue
containing approximately 10 % benzyl bromide was dried
under vacuum to yield the title product as a yellow

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solid (7.18 g, 108 %). ¹H NMR (CDCl₃) : δ 1.39 (s, 9H),
1.42 (s, 9H), 3.38-3.66 (m, 10H), 4.49 (s, 4H), 7.29 (m,
10H).

(c)   Bis(2-benzyloxyethyl)ethylenediamine

The diamine of Example 8(b) (7.18 g) was dissolved in
CH₂Cl₂ (40 mL) and cooled to 0°C. Trifluoroacetic acid
(35 mL) was added and the solution was stirred at
ambient temperature for 2 hours. After concentration,
the title product was washed with petroleum ether and
dried under vacuum. Yield : 7.2 g (93 %). ¹H NMR
(CDCl₃) : δ 7.27 (m, 10H), 4.46 (s, 4H), 3.65 (t, 4H),
3.44 (s, 4H), 3.11 (t, 4H).

(d)   Bis(chloroacetyl)-bis(2-benzyloxyethyl)-
ethylenediamine

A solution of the compound of Example 8(c) (6.81g; 20.75
mmol) and triethylamine (5.0 mL, 41.5 mmol) in CH₂Cl₂
(250 mL) was cooled to 0°C under nitrogen and
chloroacetyl chloride (3.3 mL, 41.5 mmol) was added
dropwise with stirring. The solution was stirred at
ambient temperature for 24 hours and washed with water
(7 x 20 mL), dried (MgSO₄), and concentrated. The crude
title product was purified by column chromatography on
silica gel (ether eluent) to give 2.94 g (29 %). ¹H NMR
(CDCl₃) : δ 7.28 (m, 10 H), 4.44 (m, 4 H), 4.21 (d, 4
H), 3.65-3.35 (s); 3.35 (m, 12 H).

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(e)   N,N'-Bis[1,4,7-tris-(tert-
butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecan-19-
yl-methylcarbonyl)bis(2-benzyloxyethyl)ethylenediamine

A solution of the diamine of Example 8(d) (2.94 g, 6.1
mmol), the hydrobromide salt of Example 1(b) (7.27 g,
12.2 mmol), sodium iodide (0.9 g, 6.1 mmol), and
tetramethylguanidine (2.3 mL, 18.3 mmol) in acetonitrile
(130 mL) was heated at 60°C under nitrogen for 19 hours.
The solvent was removed under vacuum and the residue was
redissolved in CH_2Cl_2 (150 mL), washed with water (100
mL), 1 M Na_2CO_3 (2 x 100 mL) and water (100 mL). The
organic layer was extracted with 1 M HCl (4 x 100 mL)
and water (100 mL). The combined aqueous layer was
basified with solid Na_2CO_3 and extracted with CH_2Cl_2 (4 x
100 mL). The organic layer was dried (Na_2SO_4) and
concentrated. The crude title product (11 g, 62 %) was
obtained as a brown solid. ^1H NMR ($CDCl_3$): δ 7.28 (m,
10 H), 4.45 (m, 8 H), 3.89-2.58 (m, 60 H), 1.45 (s, 18
H), 1.32 (s, 32 H).

(f)   N,N'-Bis[1,4,7-tris-(carboxymethyl)-1,4,7,10-
tetraazacyclododecan-10-yl-methylcarbonyl]bis(benzyloxy-
ethyl)ethylene diamine

The crude dimer of Example 8(e) (11 g) was dissolved in
CH_2Cl_2 (50 mL) and treated with trifluoroacetic acid (40
mL). The mixture was stirred at ambient temperature for
1 hour and concentrated. This process was repeated
seven times. After the final deprotection the product
was chased with CH_2Cl_2 (3 x 15 mL) and water (3 x 15 mL)
and purified by ion-exchange chromatography on BioRad
AG1 X-8 resin (100-200 mesh, acetate form), eluting with
0.1 M acetic acid. Further purification was effected by
precipitation of the product from methanol by the
addition of acetone, when the title product separated as

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a yellow solid (4.08 g; 47 %). ¹H NMR (D₂O) : δ 7.15 (s,
10 H), 4.30 (m, 4 H), 3.60 (s), 3.60-2.87 (m, 60 H).

(g)   N,N'-Bis[1,4,7-tris-(carboxymethyl)-1,4,7,10-
tetraazacyclododecan-10-yl-methylcarbonyl]bis(2-hydroxy-
ethyl)ethylenediamine

The dimer of Example 8(f) (4.05 g; 3.68 mmol) was
dissolved in a mixture of water (25 mL) and glacial
acetic acid (15 mL) and treated with Pearlman's catalyst
(2 g) and the mixture was hydrogenated in a Parr
apparatus at 52 psi until the hydrogen uptake ceased.
The solution was then filtered and concentrated. The
residue was dissolved in methanol (50 mL) and
precipitated with acetone addition (100 mL). This
process was repeated again and the title product, a pale
yellow solid was dried under vacuum. Yield : 3.07g
(88.6 %). ¹H NMR (D₂O) : δ 3.59-2.93 (m). ¹³C NMR (D₂O)
: δ 177.05; 171.74; 171.4; 169.87; 169.52; 58.52; 58.35;
56.14; 55.45; 55.28; 55.04; 52.26; 52.36; 50.97; 50.38;
49.81; 48.85; 48.59; 47.84; 44.33; 43.68; 42.97; 38.68;
37.96; 29.85.

(h)   Bisgadolinium complex of N,N'-bis[1,4,7-tris-
(carboxymethyl)-1,4,7,10-tetraazacyclododecan-10-yl-
methylcarbonyl]bis(2-hydroxyethyl)-ethylenediamine

The dimeric chelant of Example 8(g) (3.0 g, 3.26 mmol)
was dissolved in water (30 mL) and gadolinium acetate
(2.38 g, 5.86 mmol) was added. The solution was stirred
at ambient temperature for 2 hours and at 40°C for 3
hours. It was then concentrated , chased with water and
redissolved in water. The solution gave a positive
xylenol orange test. Addition of the ligand (in 1
weight % increments) followed by heating at 40°C for 1

hour was continued until the solution gave a negative
xylenol orange test. The solution was then concentrated
and chased with water (2 x 20 mL). Further purification
by precipitation from methanol/acetone solvent system (x
6) and passage through BioRad AG1-X8 resin (acetate
form) yielded the title product as a yellow solid (3.13
g, 78 %). MS (FAB): m/e: 1230 (MH$^+$). Final purification
for toxicity studies was effected by semi-preparative
HPLC on a C18 column. Elemental analysis; calculated
for $C_{38}H_{62}$ $Gd_2N_{10}O_{16} \cdot 13.1$ H_2O:
C 31.15 %, H 6.07 %, Gd 21.46 %, N 9.56 %; found: C
31.27 %, H 5.61 %, Gd 21.0 % N 9.56 % .

Example 9

**Synthesis of N,N'-bis[1,4,7-tris-(carboxymethyl)-
1,4,7,10-tetraazacyclododecan-10-yl-methylcarbonyl]-1-
(N-methylglucaminecarbonyl)- ethylenediamine and
bisgadolinium complex thereof**

(a)   N-BOC-N-methylglucamine

N-methylglucamine (4.0 g; 20.49 mmol) and di-t-butyl
dicarbonate (4.48 g; 20.51 mmol) were dissolved in
methanol (40 mL) and the solution was stirred under
nitrogen for 24 hours. Concentration and washing with
petroleum ether yielded the title product as a colorless
solid (6.48 g, 100 %). ^{1}H NMR (D_2O) : δ 3.59-3.38 (m, 6
H), 3.1 (s, 2 H), 2.67 (br, 3 H), 1.21 (s, 9 H).

(b)   N-BOC-N-methyl-pentakis-(O-benzyl)glucamine

An 80 % suspension of sodium hydride (1.5 g, 50 mmol)
was washed with tetrahydrofuran (20 mL) under nitrogen.

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Benzyl bromide (17.8 mL, 150 mmol) and tetrahydrofuran
(30 mL) were added followed by N-BOC-N-methylglucamine
(Example 9(a), 2.95 g, 10 mmol). The mixture was
stirred at ambient temperature under nitrogen overnight.
The reaction was quenched by the gradual addition of
water (30 mL) with stirring. The organic layer was
separated and the aqueous layer extracted with CH_2Cl_2 (2
x 30 mL). The combined organic extract was washed with
water, dried ($MgSO_4$) and concentrated to obtain a yellow
oil from which the excess benzyl bromide was distilled
off under vacuum at a bath temperature of 100°C. The
crude title product was obtained as a sticky yellow
solid (7 g; 94 %). ^1H NMR ($CDCl_3$) : δ 7.38 (m, 25 H),
4.79-4.44 (m, 10 H), 4.04-3.76 (m, 6 H), 3.49 (m, 2 H),
2.96 (s), 2.78 (d, 3 H), 1.48-1.44 (d, 9 H). ^{13}C NMR
($CDCl_3$) : 155.72; 138.53 (m); 127.68 (m); 79.0-76.49 (m);
73.93-69.60 (m); 49.96; 35.94; 28.41.

(c)   N-Methyl-pentakis(O-benzyl)glucamine

The crude glucamine of Example 9(b) (7.02 g, 9.43mmol)
was dissolved in CH_2Cl_2 (25 mL) and the solution was
cooled in an ice bath. Trifluoroacetic acid (40 mL) was
added and the mixture was stirred at ambient temperature
for 2 hours. The solution was concentrated and the
deprotection process was repeated again. The solution
was chased with CH_2Cl_2 (2 x 15 mL), washed with saturated
$NaHCO_3$ (30 mL), water (30 mL), dried (Na_2SO_4), and
concentrated to yield the title product as a brown oil
which was purified by chromatography on silica gel using
5 % methanol in chloroform as the eluent. Yield : 5.55
g (91 %). ^1H NMR ($CDCl_3$) : δ 7.33 (m, 25 H), 4.8-4.41
(m, 10 H), 4.06-3.61 (m, 6 H) 3.03-2.71 (m, 2 H), 2.41
(s, 3 H). ^{13}C NMR ($CDCl_3$) : δ 137.97 (m); 128.25;
78.85-69.55 (m); 50.41; 39.94.

(d)   N,N'-Bis(BOC)-2,3-diaminopropionic acid

A suspension of diaminopropionic acid hydrochloride
(5.13 g, 36.5 mmol) in a mixture of ethanol (70 mL) and
methanol (20 mL) was treated with triethylamine (10.2
mL, 73 mmol), diisopropylethylamine (5 mL) and di-t-
butyldicarbonate (16.72 g, 76.6 mmol). The mixture was
stirred at ambient temperature overnight and refluxed
for 7 hours under nitrogen. The solution was filtered
and concentrated. The residue was washed with petroleum
ether (3 x 20 mL), treated with chloroform (150 mL) and
the mixture cooled to 0°C. 1 M H_2SO_4 (150 mL) was added
and the mixture was stirred at 0°C for 15 minutes. The
aqueous layer was removed and extracted with chloroform
(2 x 30 mL). The combined organic layers were washed
with water, dried (Na_2SO_4) and concentrated to obtain the
title product as a colorless solid (9.57 g; 86.3 %). ^{1}H
NMR (CDCl$_3$) : δ 7.34 (br, 1 H), 6.25-5.19 (br, 2 H), 4.3
(br, 1 H), 3.52 (m, 2 H), 1.42 (s, 18 H). ^{13}C NMR
(CDCl$_3$) : δ 173.32; 54.69; 42.22; 28.29.

(e)   N,N'-Bis(BOC)-(N-methyl-pentakis-O-benzyl-glucaminecarbonyl)-ethylenediamine

A mixture of the propionic acid of Example 9(d) (2.24 g,
7.37 mmol), dicyclohexylcarbodiiimide (1.52 g, 7.37
mmol), the glucamine of Example 9(c) (4.76 mg, 7.37
mmol) and dimethylaminopyridine (0.09 mg, 0.74 mmol) in
CH_2Cl_2 (50 mL) was stirred at ambient temperature under
nitrogen overnight. The solution was filtered, washed
with water, dried (MgSO$_4$) and concentrated to give a
brown oil. Purification by column chromatography on
silica gel (chloroform eluent) yielded the title product
(6.19 g; 90 %). ^{1}H NMR (CDCl$_3$) : δ 7.32 (m, 25 H), 4.81-
4.65 (m, 10 H), 4.04-3.75 (m, 4 H), 1.45 (d, 18 H). ^{13}C
NMR (CDCl$_3$) : δ 170.17, 169.92, 155.8, 155.2, 138.21,

127.76, 79.46-76.49 (m), 74.30-69.21 (m), 50.78-48.65
(m), 42.59, 36.31, 28.15, 28.1, 25.46, 24.81.

(f)   N-methyl-pentakis(O-benzyl)glucamine-carbonylethylenediamine

The ethylenediamine of Example 9(e) (5g) was dissolved
in CH_2Cl_2 (60 mL) and the solution was cooled to 0°C.
Trifluoroacetic acid (60 mL) was added and the solution
was stirred at ambient temperature for 2 hours. The
solution was concentrated and the process was repeated
again. After concentration the residue was chased with
CH_2Cl_2 (3 x 15 mL), dissolved in $CHCl_3$, and washed with
saturated $NaHCO_3$ (2 x 30 mL) and water and dried ($MgSO_4$).
The title product was obtained as a brown oil after
concentration (4.72 g). ^1H NMR ($CDCl_3$) : δ 7.3 (m, 25
H), 4.76-4.25 (m, 11 H), 3.96-3.41 (m, 10 H), 2.94-2.52
(m, 3 H). ^{13}C NMR ($CDCl_3$) : δ 138.62, 127.7, 78.89-76.5
(m), 74.63-69.55 (m), 53.62-46.0 (m), 39.62-24.90. MS
(FAB) : m/e: 732.6 (MH⁺).

(g)   N-Methyl-pentakis(O-benzyl)glucaminecarbonyl-ethylenediamine-bis(chloroacetamide)

The ethylenediamine of Example 9(f) (3.9 g, 5.33 mmol)
and triethylamine (1.5 mL, 10.7 mmol) were dissolved in
chloroform (50 mL) and the solution was cooled to 0°C
under nitrogen. Chloroacetyl chloride (1.2 g, 10.6 mmol)
was added slowly. After the addition was complete the
solution was warmed to ambient temperature and stirred
overnight. The solution was washed with water (3 x 20
mL), dried ($MgSO_4$) and concentrated. The residue was
chromatographed on silica gel, eluting with
methanol/chloroform solvent mixture (0-5 %) to yield the
title product 3.82 g (81 %). ^1H NMR ($CDCl_3$) : δ 7.30 (m,

25 H), 4.78-4.40 (m, 10 H), 4.30 (m, 1 H), 4.01-3.19 (m, 14 H), 2.94-2.51 (m, 3 H). ^{13}C NMR (CDCl$_3$) : δ 168.85-165.55 (m), 137.98-137.5 (m), 127.86-126.74 (m), 78.29-75.55 (m), 74.09-71.38 (m), 68.94-68.72 (m), 49.08-48.64 (m), 42.14-40.99 (m), 36.5; 35.8, 33.76-33.1, 25.07, 24.41. MS (FAB): m/e 884.4 (MH$^+$).

(h)   N,N'-Bis[1,4,7-tris-(tert-butoxycarbonyl-methyl)-1,4,7,10-tetraazacyclododecan-10-yl-methyl-carbonyl]-N-methyl-pentakis(O-benzyl)glucamine-carbony-ethylenediamine

The hydrobromide salt of Example 1(b) (5.12 g, 8.6 mmol) and the bis(chloroacetamide) of Example 9(g) (3.8 g, 4.3 mmol) were dissolved in CH$_3$CN (100 mL) and tetramethylguanidine (1.62 mL, 12.9 mmol) was addded. The solution was heated at 60°C under nitrogen for 25 hours. After concentration, the residue was taken up in chloroform and washed with water and dried (MgSO$_4$). Following concentration, the crude title product was obtained as a viscous yellow material (9.08 g). ^{1}H NMR (CDCl$_3$) : δ 7.22 (m, 25 H), 4.70-4.32 (m, 11 H), 4.00-3.67 (m, 6 H), 3.38-2.49 (m, 55 H), 1.39-1.35 (m, 54 H). ^{13}C NMR (CDCl$_3$) : δ 174.27 -169.56 (m), 138.5-136.6 (m), 132.49-127.11 (m), 81.06-80.16 (m), 78.62-76.49 (m), 73.62-69.37(m), 57.62-47.28 (m), 36.9; 27.97. MS (FAB): m/e 1842.1 (MH$^+$).

(i)   N,N'-Bis[1,4,7-tris-(carboxymethyl)-1,4,7,10-tetraazacyclododecan-10-yl-methylcarbonyl]-N-methyl-pentakis(O-benzyl)glucamine-carbonylethylenediamine

The dimer of Example 9(h) (7.9 g, 4.29 mmol) was dissolved in dichloromethane (80 mL) and cooled to 0°C. Trifluoroacetic acid (80 mL) was added and the solution was stirred at ambient temperature for 1 hour. The

solution was concentrated and the process was repeated
nine times. After the final deprotection, the solution
was concentrated and chased with CH_2Cl_2 (4 x 20 mL) and
water (4 x 20 mL). Drying under vacuum yielded the
crude title product (10.9 g). ^{1}H NMR (D_2O) : δ 6.53
(br, 25 H), 4.08-2.37 (m, 72 H). ^{13}C NMR (D_2O): δ
126.33, 52.33, 51.05, 50.13, 47.27, 45.68, 40.27. MS
(FAB): m/e 1504.5 (MH$^+$).

(j)   N,N'-Bis[1,4,7-tris-(carboxymethyl)-1,4,7,10-
tetraazacyclododecan-10-yl-methylcarbonyl]-1-(N-methyl-
glucaminecarbonyl)-ethylenediamine

A solution of the dimer of Example 9(i) (0.86 g, 0.57
mol) in water (20 mL) was treated with glacial acetic
acid (5 mL) and Pearlman's catalyst (0.5 g). The
mixture was hydrogenated in a Parr hydrogenation
apparatus with hydrogen gas at a pressure of 53 psi
until no further uptake of hydrogen was observed. The
solution was filtered, concentrated and chased with
water. The residue was purified by ion exchange
chromagraphy using BioRad AG1-X8 resin (100-200 mesh,
acetate form) using acetic acid as the eluent (0.05-0.2
M) to give 0.42 g (70 %) of the title product as a pale
yellow solid. ^{1}H NMR (D_2O) : δ 3.8 -2.66 (m). ^{13}C NMR
(D_2O): δ 175.17, 179.46, 172.17-170.77, 72.22-70.61,
63.31, 56.87-48.77, 40.59-35.18. MS (FAB): m/e 1054.6
(MH$^+$).

(k)   Synthesis of Bisgadolinium complex of N,N'-
bis[1,4,7-tris-(carboxy-methyl)-1,4,7,10-tetraazacyclo-
dodecan-10-yl-methylcarbonyl]-1-(N-methylglucamine-
carbonyl)ethylenediamine

To a solution of the dimeric chelant of Example 9(j)

(0.94 g, 0.89 mmol) in water (25 mL) was added
gadolinium acetate (0.47 g) and the solution was stirred
at 40°C overnight. It was concentrated and the solution
was repeatedly chased with water until the pH was to
5.2. The xylenol orange test for free gadolinium was
negative. Further quantities of gadolinium acetate were
added in 1 weight % increments and the solution heated
at 40°C for 1 hour until the reaction mixture showed a
postive xylenol orange test for gadolinium. The
solution was filtered, concentrated, and chased with
water. The crude title product was subjected to
purification by semi-preparative HPLC on a C18 column
using 2 % methanol in water, followed by precipitation
from a methanol solution with acetone to give 0.78 g (52
%). MS (FAB): m/e 1364.3 (MH$^+$).

Example 10

Synthesis of bis[1,4,7-tris-(carboxymethyl)-1,4,7,10-
tetraazacyclododecan-10-yl]-2-oxo-3-aza-pentane and the
gadolinium and dysprosium complexes thereof

(a)   3-Aza-4-oxo-1,5-dibromopentane

Bromoethylamine hydrobromide (6.15 g, 30 mmol) was
suspended in chloroform (40 mL) and treated with
diisopropylethylamine (5.2 mL, 60 mmol). The solution
was cooled in a dry ice acetone bath and a solution of
bromoacetyl bromide (2.6 mL, 30 mmol) in chloroform (10
mL) was added dropwise under nitrogen. After the
addition was complete, the solution was warmed to
ambient temperature and stirred overnight. The solution
was washed successively with water (2 x 30 mL), 1 M

acetic acid (2 x 30 mL), water (30 mL), 1 M NaOH (2 x 30 mL) and water (2 x 30 mL). Drying over anhydrous Na_2SO_4 followed by concentration yielded the crude product as a colorless solid (4.94 g; 66 %). Recrystallization from chloroform yielded the pure title product (1.33 g, 18.1 %). ^1H NMR ($CDCl_3$): δ 6.84 (br, 1 H), 3.91 (s, 2 H), 3.72 (q, 2 H), 3.5 (t, 2 H). ^{13}C NMR ($CDCl_3$) : δ 166.08, 41.54, 31.11, 28.67.

(b)   Bis[1,4,7-tris-(tert-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecan-10-yl]-2-oxo-3-azapentane

3-Aza-4-oxo-1,5-dibromopentane (Example 10(a), 1.28 g, 5.22 mmol), the hydrobromide salt of Example 1(b) (6.22 g, 10.44 mmol) and tetramethylguanidine (2 mL, 15.66 mmol) were dissolved in acetonitrile (125 mL) and the solution was heated at 60°C for 48 hours under nitrogen. The solution was concentrated, the residue was dissolved in chloroform and washed with water. It was dried over anhydrous Na_2SO_4 and concentrated to yield a brown oil. Petroleum ether extraction followed by concentration of the extract yielded the crude title product (6.26 g). ^1H NMR ($CDCl_3$): 8.8 (br, 1 H), 3.46-2.38 (m, 50 H), 1.28 (s, 54 H). ^{13}C NMR ($CDCl_3$) : δ 172.2-169.84, 80.47, 61.83-47.37, 27.94, 27.9. MS (FAB): m/e 1112.9 (MH^+).

(c)   Bis[1,4,7-tris-(carboxymethyl)-1,4,7,10-tetraazacyclododecan-10-yl]-2-oxo-3-aza-pentane

To a solution of the dimer of Example 10(b) (6.25 g) in CH_2Cl_2 (60 mL) cooled in an ice bath, is added trifluoroacetic acid (60 mL) and the mixture stirred at ambient temperature for 1 hour. The solution is concentrated and the process is repeated nine times.

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After the final deprotection, the solution is concentrated and chased with CH_2Cl_2 (3 x 20 mL) and water (3 x 20 mL). The residue is passed through BioRad AG1 X-8 ion exchange resin (100-200 mesh, acetate form) and the product eluted with aqueous acetic acid (0.05-0.1 M acetic acid).

(d)   Bisgadolinium complex of bis[1,4,7-tris-(carboxymethyl)-1,4,7,10-tetraazacyclododecan-10-yl]-2-oxo-3-aza-pentane

The dimeric chelant of Example 10(c) is dissolved in water and gadolinium acetate is added. The solution is stirred at 40°C for 2 hours. The pH of the solution is raised from 3 to 5 by adding 0.1 M ammonium hydroxide followed by chasing with water. Addition of gadolinium acetate in 1 wt. % increments and heating at 40°C is continued until a positive xylenol orange test is observed. The solution is then filtered and concentrated. The residue is chased with water and dried under vacuum to obtain the title product.

(e)   Bis dysprosium complex of bis[1,4,7-tris-(carboxymethyl)-1,4,7,10-tetraazacyclododecan-10-yl]-2-oxo-3-aza-pentane

The dysprosium complex is prepared analogously to Example 10(d) using the dimeric chelant of Example 10(c) and a soluble dysprosium(III) salt.

Example 11 - Experimental Results

Gadolinium complexes of a series of dimeric substituted
tetraazacyclododecane macrocycles (Table 1) have been
synthesized. Their physicochemical properties were
studied to evaluate their utility as extracellular fluid
MRI contrast agents. The results are presented here.

Experimental

The D03A bis(amide) dimer ligands 1-11 shown in Table 1
were prepared by the coupling reaction of D03A-tri-t-
butyl ester with bis(chloroacetamides) of the
appropriate diamines, followed by the deprotection of
the t-butyl ester groups. The gadolinium complexes were
prepared by the reaction of the ligands with Gd(OAc)$_3$.
Relaxivities were measured in water and in serum (in
selected cases) at 40°C and 20 MHz. Viscosities and
osmolalities were measured at concentrations listed in
Table 2.

Conclusion

The favourable physicochemical profiles of the dimeric
gadolinium chelates presented here, which include
relaxivity, viscosity and osmolality suggest their
potential use as new extracellular fluid MRI contrast
agents.
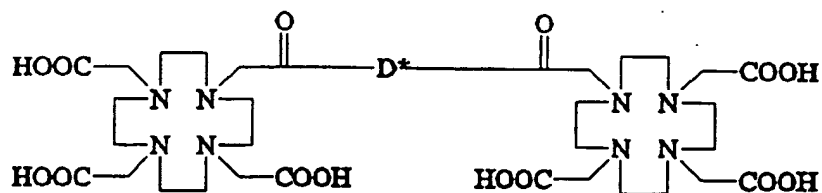
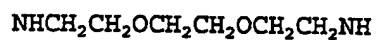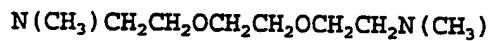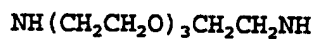| Compound No.	D*
1	$N(CH_3)CH_2CH_2N(CH_3)$
2	$N(CH_2CH_2OH)CH_2CH_2N(CH_2CH_2OH)$
3	$N(CH_2CHOHCH_2OH)CH_2CH_2N(CH_2CHOHCH_2OH)$
4	$NHCH(CON(CH_3)CH_2(CHOH)_4CH_2OH)CH_2NH$
5	
6	
7	$NHCH_2CH_2OCH_2CH_2NH$
8	$NHCH_2CH_2OCH_2CH_2OCH_2CH_2NH$
9	$N(CH_3)CH_2CH_2OCH_2CH_2OCH_2CH_2N(CH_3)$
10	$NH(CH_2CH_2O)_3CH_2CH_2NH$
11	

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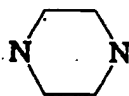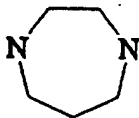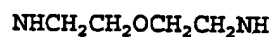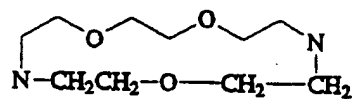
Table 2 - Physicochemical properties of complexes

Complex	Relaxivity[a] (mM^{-1} sec^{-1} Gd^{-1})		Osmolality[b] (mmol/kg)	Viscosity[c] (cPs) 25°C 37°C	
	r_1	r_2			
1	5.6	5.5	323 (200)	2.0	1.4
2	5.2	4.9	555 (200)	2.1	1.5
3	5.5	6.2	345 (275)	.[d]	.[d]
4	6.1	6.9	1083 (384)	.[d]	.[d]
5	5.8	6.6	581 (250)	3.0	2.0
6	5.1	5.9	474 (250)	2.6	1.8
7	4.7	4.8	653 (314)	2.8	2.1
8	4.7	5.7	752 (308)	3.3	2.4
9	5.0	6.5	635 (278)	2.7	2.0
10	4.4	5.3	793 (281)	3.4	2.6
11	5.7	7.1	971 (257)	2.9	2.1

[a] in water at 40°C and 20 MHz

[b] concentrations (mM) in parentheses

[c] same concentrations as in osmolality unless otherwise stated

[d] not measured

Claims

1. Dichelants of formula V
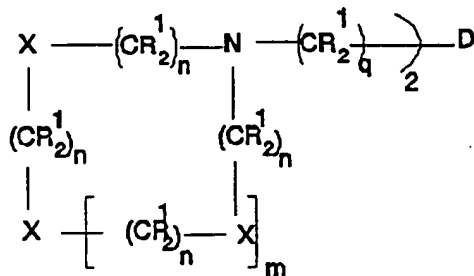
(wherein each X which may be the same or different is
NZ, O or S, at least two Xs being NZ;
each Z is a group R^1 or a group CR^1_2Y, at least one Z, on
each macrocyclic ring being a group CR^1_2Y;
each Y is a group CO_2H, PO_3H, SO_3H, $CONR^1_2$, $CON(OR^1)R^1$,
CNS or $CONR^1NR^1_2$;
m is 0 or 1 or 2;
each n is 2 or 3;
q is 1 or 2;
each R^1 which may be the same or different is a hydrogen
atom or an alkyl group optionally substituted by one or
more hydroxy and/or alkoxy groups;
and D is a bridging group, other than an unsubstituted
carbonylaminoethylaminocarbonyl group, having a
molecular weight of less than 1000 joining two
macrocyclic rings via at least one amide or ester bond)
or salts or metal chelates thereof.

2. Compounds as claimed in claim 1 wherein each
macrocyclic $(CR^1_2)_n(X(CR^1_2)_n)_{m+2}N$ ring has 9 to 14 ring
atoms.

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3.   Compounds as claimed in either of claims 1 and 2
wherein in each said macrocyclic ring each n is 2.

4.   Compounds as claimed in any one of claims 1 to 3
wherein bridging group D has a molecular weight of less
than 500.

5.   Compounds as claimed in any one of claims 1 to 4
wherein each q is 1.

6.   Compounds as claimed in any one of claims 1 to 5
wherein each m is 1.

7.   Compounds as claimed in any one of claims 1 to 6
wherein each y is a group COOH or $COO^{\ominus}$.

8.   Compounds as claimed in any one of claims 1 to 7
wherein bridging group D is of formula
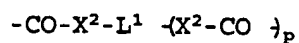
$$-CO-X^2-L^1 \;\; (X^2-CO \;)_p$$

where p is 0 or 1,
X^2 is O or NR^2,
R^2 is a hydrogen atom or a hydroxy, OR^1 or NR^1_2 group or
an alkyl group optionally interrupted by oxygen, sulphur
or nitrogen atoms or by carbonyl or aryl groups and
optionally substituted by hydroxyl, amine or aryl
groups, or R^2 contains a functional group for attachment
to a biomolecule or macromolecule, or two R^2 groups
together form a bridging linker group, and L^1 which
provides a chain or at least two atoms linking two X^2
groups or at least one atom linking an X^2 group and a
$(CR^1_2)_q$ moiety (wherein R^1 and q are as defined in claim
1), is a straight chain, branched or cyclic alkylene
group or a combination of such groups, optionally
substituted and optionally being interrupted by oxygen,
sulphur or nitrogen atoms or by aryl or carbonyl groups.

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9. Compounds as claimed in any one of claims 1 to 8 wherein D is of a formula selected from the group consisting of

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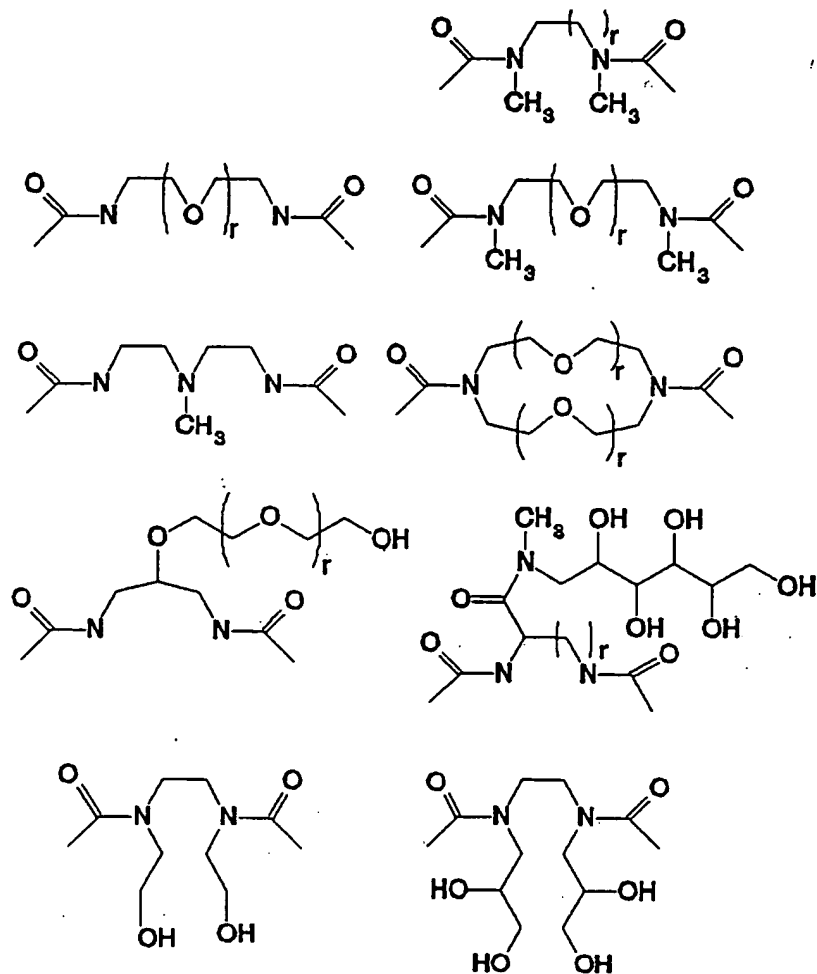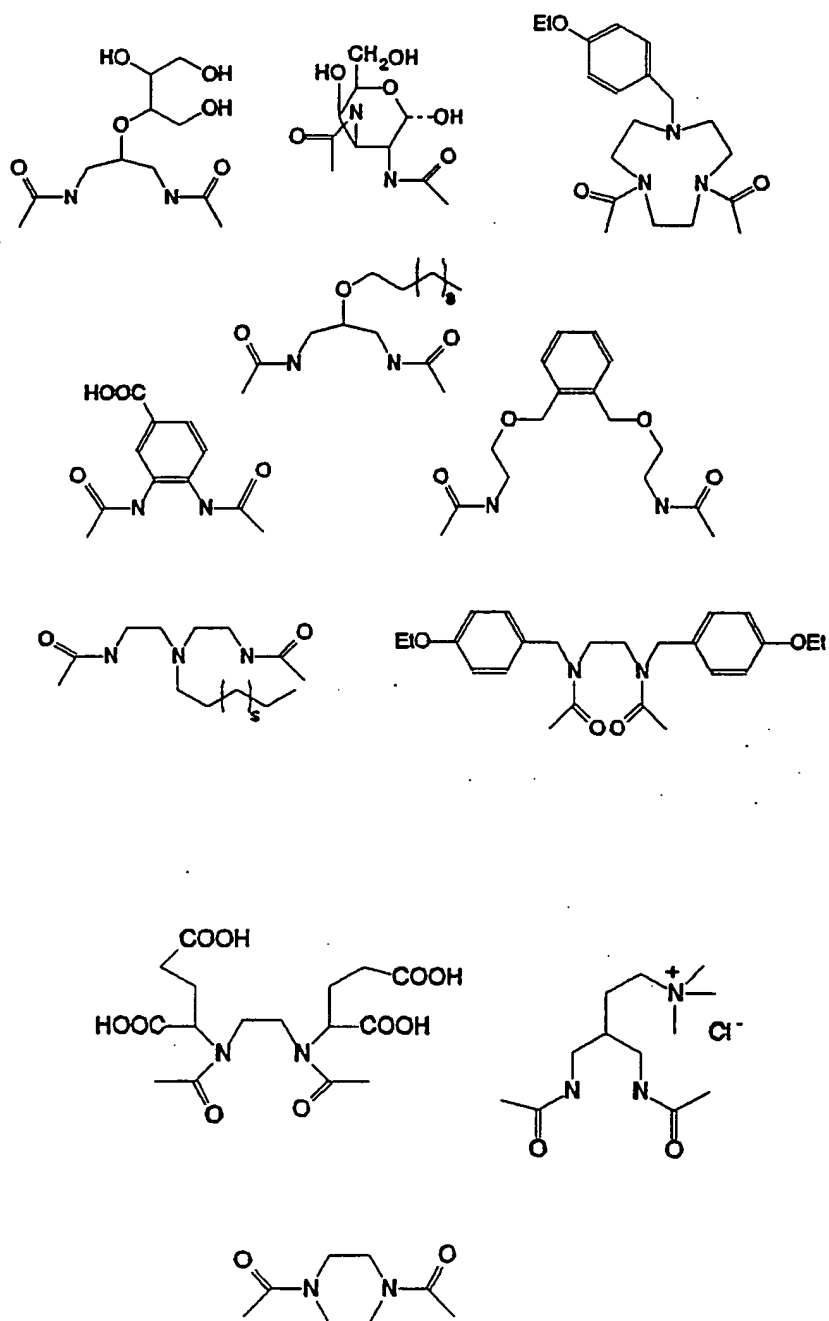
where r is an integer having a value of 1 to 6, and s is
an integer having a value of 1 to 20.

10.   Compounds as claimed in any one of claims 1 to 8
wherein D is of formula $COOCH_2CH_2OCH_2CH_2NHCH_2CH_2OCH_2CH_2OCO$.

11.   Compounds as claimed in any one of claims 1 to 8
wherein D is of formula $CONHCH_2CH_2OCH_2CH_2OCH_2CH_2NHCO$.

12.   Compounds as claimed in claim 1 being chelants of
formula VII

$$M-CH_2CO)_2-D' \qquad\qquad (VII)$$

where M is a nitrogen attached triaza, tetraaza,
triazaoxa or triazathia-cycloalkane having at least one
and preferably two ring nitrogens substituted by CH_2COOH
groups and having any remaining ring nitrogen
substituted by a group R^3;
R^3 is a hydrogen atom, or an alkyl group optionally mono
or polysubstituted by hydroxyl or alkoxy groups and
optionally interrupted by arylene or substituted arylene
groups; and
$CO-D'-CO$ is a bridging group as defined for D in claim 1
and salts and metal chelates thereof.

13.   A process for the preparation of compounds as
claimed in claim 1 said process comprising at least one
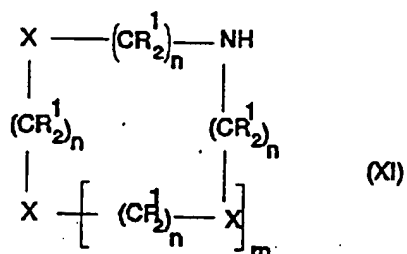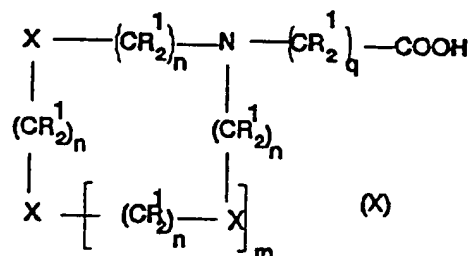of the following steps:

(a) reacting a compound of formula V wherein at least
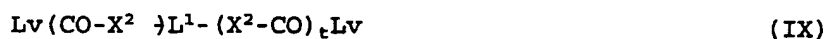one group X is a group NH, with a compound of formula
VIII

$$Lv-R^4 \qquad (VIII)$$

(where Lv is a displaceable leaving group and R^4 is a

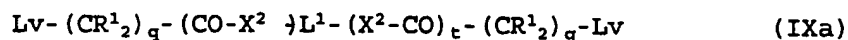group CR^1_2Y or group R^1 other than hydrogen);

(b) reacting compounds of formulae X and/or XI

(X)

(XI)

(wherein X, R^1, n, q and m are as defined in claim 1) or
an activated derivative thereof with a linker molecule
of formula IX

$$Lv(CO-X^2 \;)L^1-(X^2-CO)_t Lv \qquad\qquad (IX)$$

or formula IXa

$$Lv-(CR^1_2)_q-(CO-X^2 \;)L^1-(X^2-CO)_t-(CR^1_2)_q-Lv \qquad (IXa)$$

(wherein R^1 and q are as defined in claim 1 and X^2 and L^1
are as defined in claim 8, t is 0 or 1, and each Lv is a
displaceable leaving group, one optionally being
protected prior to conjugation of the second compound of
formula X or XI);

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(c) converting a compound of formula X into a corresponding acid chloride and reacting with a suitable diamine;

(d) metallating or transmetallating a compound of formula V or a chelate thereof;

(e) converting a compound of formula V or a chelate thereof into a base or acid addition salt thereof or converting a salt into the free acid or base; and

(f) performing at least one of steps (a) to (d) above using reagents with protected functional groups and subsequently removing the protecting groups.

14. A method of generating enhanced images of the human or non-human animal body, which method comprises administering to said body an effective amount of a diagnostic agent comprising a metal chelate of compound of formula V as defined in claim 1 or a salt thereof, and generating an image of at least part of said body to which said chelate distributes, wherein said metal is paramagnetic, radioactive or X-ray opaque.

15. A method of radiotherapy practised on the human or non-human animal body, which method comprises administering to said body an effective amount of a chelate of a radioactive metal species with a chelating agent of formula V as defined in claim 1 or a salt thereof.

16. A method of heavy metal detoxification practised on the human or non-human animal body, which method comprises administering to said body an effective amount of a chelating agent of formula V as defined in claim 1 or a physiologically tolerable salt or weak complex thereof.

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17. A process for the preparation of the metal chelates
of the invention which process comprises admixing in a
solvent a compound of formula V as defined in claim 1 or
a salt or chelate thereof together with an at least
sparingly soluble compound of said metal.